DOCTOR OF PHILOSOPHY

Evaluating the use and effectiveness of environmental enrichments in intensive broiler housing

Baxter, Mary

Award date:
2018

Awarding institution:
Queen's University Belfast

Evaluating the use and effectiveness of environmental enrichments in intensive broiler housing

Mary Sheila Stella Baxter

BSc (Hons) in Bioveterinary Science, Liverpool University

MSc in Applied Animal Behaviour and Welfare, Edinburgh University

A thesis submitted for the degree of Doctor of Philosophy in the Institute for Global Food Security

School of Biological Sciences, Queens University Belfast

16th February 2018

Acknowledgments

I am extremely grateful to Dr Niamh O'Connell for her generous supervision, constant encouragement, and for always leaving me feeling inspired after our meetings.

This PhD was supported by the Department for Employment and Learning in Northern Ireland and from a grant received from the World's Poultry Science Association – UK branch. I have relied heavily on the kindness and openness of the farmers that hosted these experiments, especially Stephen Bell who adopted a clueless first year student. I am grateful to the staff at Moy Park Ltd for their support in facilitating these trials and for their helpful advice. My thanks also go to my amazing friends and colleagues, for their kindness, wisdom and camaraderie. I am particularly indebted to Grace, Poppy, Matt and Ellie, who have kept me sane.

I dedicate this thesis to my father, who built me my first chicken shed and let me create a bigger and bigger family of pet chickens with gentle resignation. He helped foster a lifelong interest in their welfare and I hope he would have been proud. To my brother, who constantly asked when I was getting a real job but can recite my thesis aims to anyone that asks. To my incredible mother, who has been my therapist, long-suffering proof reader and unwavering cheer-leader. It's always nice to hear that I am literally the best scientist in the world.

To Peter, for your love. These years have been worth it for that alone.

Lastly, to the broilers that sat on my feet and kept me company while I worked, I hope this helps.

"The worst sin towards our fellow creatures is not to hate them, but to be indifferent to them: that's the essence of inhumanity."

~ George Bernard Shaw, *The Devil's Disciple*

List of publications from thesis

Baxter, M., Bailie, C. L., and O’Connell, N. E. (2017). An evaluation of potential dustbathing substrates for commercial broiler chickens. Animal. Advanced online publication. https://doi.org/10.1017/S1751731117003408

Baxter, M., Bailie, C. L., & O’Connell, N. E. (2018). Evaluation of a dustbathing substrate and straw bales as environmental enrichments in commercial broiler housing. Applied Animal Behaviour Science, 200, 78-85. http://dx.doi.org/10.1016/j.applanim.2017.11. 010

Baxter, M., Bailie, C. L., and O’Connell, N. E. (accepted). Play behaviour, fear responses and activity levels in commercial broiler chickens provided with preferred environmental enrichments. Animal.

Baxter, M. and O’Connell, N. E. (under review). Do commercial broiler chickens use environmental enrichments differently when they are grouped together rather than provided singly?

Baxter, M., Bailie, C. L., and O’Connell, N. E. (2017). Comparison of foraging and dustbathing enrichments for commercial broiler chickens. British Poultry Abstracts Vol. 13: WPSA Annual Spring Meeting UK Branch, 2017. Abingdon, UK: Taylor & Francis, pp. 1-39.

Baxter, M. and O’Connell, N. E. (2016). Commercial comparison of potential dustbathing substrates for intensively farmed broilers. Book of abstracts of the 67th Annual Meeting of the European Association for Animal Production. EAAP Scientific Community (Eds). The Netherlands: Wageningen Academic Publishers, pp. 398.

Additional publication by the author

Bailie, C. L., Baxter, M., and O’Connell, N. E. (2018). Exploring perching provision options for commercial broiler chickens. Applied Animal Behaviour Science, 200, 78-85. doi.org/10.1016/j.applanim.2017.12.007.

Abstract

The main aim of this research was to determine whether broiler welfare would be improved by the addition of a dustbathing material to commercial housing. An initial comparison of potential dustbathing materials in Study 1 found an expected preference for peat, however oat hulls also appeared to satisfy broilers motivation to dustbathe and proved considerably more attractive than straw pellets, woodshavings and litter. In Study 2, dust baths of oat hulls were introduced to commercial housing as an alternative or supplementary enrichment to straw bales. Houses containing oat hulls were compared with those containing straw bales, a combination of straw bales and oat hulls, or no enrichment. Although there was no effect of any enrichment condition on house activity levels, there was an improvement in gait score in broilers housed with both oat hulls and a combination of oat hulls and straw bales. Oat hulls were more successful than straw bales at directly stimulating active foraging and dustbathing behaviours, however the bales appeared to provide birds with a valuable resting area and were dismantled throughout the trial. There was also no negative impact of these enrichments on environmental parameters or production levels, including bird body weight. With oat hulls appearing to be a suitable supplementary enrichment, there was interest in knowing how best to present multiple enrichments. Therefore, in Study 3, oat hulls, pecking chain and straw bales were presented singly or arranged into various combinations around a commercial house. The number of broilers attracted to the enrichment areas and the level of engagement with each enrichment type was monitored. There was little effect of grouping enrichments on their level of use, and placing straw bales around oat hulls did not influence the amount of dustbathing and comfort behaviours observed. In fact, there appeared to be practical benefits to distributing enrichments around the house. Study 4 was designed to explore the effects of environmental enrichment on broiler experience and mental well-being. Frequency of stimulated play behaviours and strength of fear responses were compared in houses containing no enrichment, platform perches, and platform perches with peat dust baths. Although no difference in play behaviours was found between treatments, the method of stimulating play described may prove useful in further examining the relevance of these behaviours. Fearfulness appeared to be mitigated in houses containing dust baths, which suggests providing broilers

with the opportunity to dustbathe may influence their mental state in commercial housing.

This thesis has provided an original contribution to animal welfare research by studying the potential benefits of providing a dustbathing enrichment to commercial broiler chickens, and by describing a novel method of stimulating frolicking and sparring behaviours which may be useful in further understanding play in poultry. This research has also highlighted the need for more commercial scale research for broiler chickens, for example a higher interest in a pecking enrichment was observed in this thesis than has been reported previously. Oat hulls, which are a by-product of oat milling, are suggested as a suitable dustbathing material for broilers in intensive housing. Further research exploring the most efficient ways of presenting and maintaining oat hulls in a commercial house would be useful, and an assessment of their effect on dust levels would be needed to ensure no risk to farm workers.

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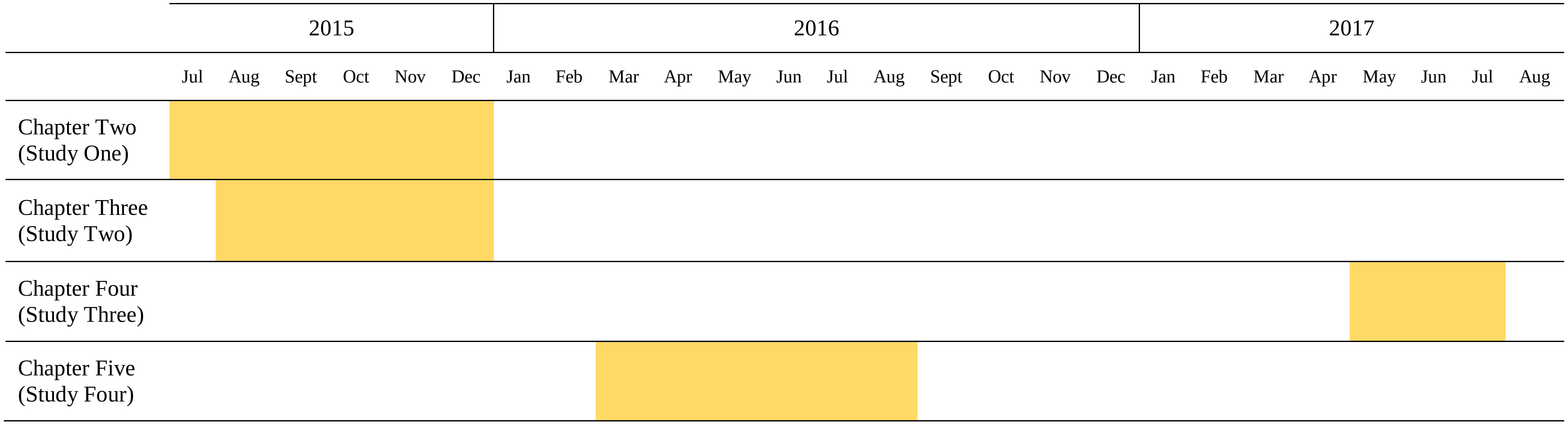
Progression of research



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Chapter One

Introduction

1.1 Attitudes to farm animal welfare

An increase in public awareness of environmental and animal welfare issues is usually attributed to the publication of two 20th century books, Silent Spring by Rachel Carson (1962) and Animal Machines by Ruth Harrison (1964). With a larger proportion of the population living in towns after the war and with less insight into farming practices, these books had international impact and a UK government committee was immediately set up to investigate farming standards (Appleby, 2003; Keeling, 2005). The committee published the Brambell Report in 1965 which confirmed that animal welfare in intensive systems was regularly compromised. The recommendations that were published from this report are a stark confirmation of the severe failure of intensive housing to provide an adequate environment at the time; the report stated that "*an animal should at least have sufficient freedom of movement to be able without difficulty, to turn round, groom itself, get up, lie down and stretch its limbs".* Following this report, the independent Farm Animal Welfare Council (later the Farm Animal Welfare Committee; FAWC) was established to advise the government. FAWC built on the Brambell Report and developed the internationally acknowledged 'Five Freedoms', which outlined the minimum requirements for farmed animals (FAWC 1992):

1. **Freedom from Hunger and Thirst** - by ready access to fresh water and a diet to maintain full health and vigour.
2. **Freedom from Discomfort** - by providing an appropriate environment including shelter and a comfortable resting area.

3. **Freedom from Pain, Injury or Disease** - by prevention or rapid diagnosis and treatment.
4. **Freedom to Express Normal Behaviour** - by providing sufficient space, proper facilities and company of the animal's own kind.
5. **Freedom from Fear and Distress** - by ensuring conditions and treatment which avoid mental suffering.

The Five Freedoms are not without criticism, largely because they focus heavily on reducing suffering rather than providing animals with ‘a life worth living’ (Yeates and Main, 2008; FAWC, 2009; McCulloch, 2013). However, they have been used extensively to identify problems in intensive systems and are the basis for common welfare regulations. Since the Brambell Report, there have been improvements to various aspects of intensive farming in the UK, for example in space allowances, housing conditions and feeding strategies. Nevertheless, considerable welfare issues remain and animal welfare continues to be of interest to the general public. An EU survey in 2006 found that when asked to score the importance of animal welfare on a scale of 1-10, the average score from all respondents was 7.8 (EU Commission, 2006). When a similar survey was repeated in 2015, asking respondents if they felt protecting the welfare of farmed animals was important, 94% of respondents said that it was (EU Commission, 2015).

Although the UK has often led the way in making improvements to farming systems, a plateau in these advances has been reported (FAWC, 2009). The UK has resisted “gold-plating” EU legislation and there has been a stalling on improvements to well-established issues, such as lameness in dairy cows and broilers, slaughter without stunning, and stocking densities. There also remains persistent reliance on legal mutilations in some cases, and an absence of sufficient labelling schemes to allow consumers to choose higher welfare options (FAWC, 2009). The UK is now in a novel position of being able to re-write the majority of its laws following its imminent departure from the European Union. Approximately 80% of UK laws related to animal welfare originate from the EU (UK Parliament, 2017), and an RSPCA survey found that 81% of those polled would want these laws to be improved or stay the same after Brexit (RSPCA, 2017a). Although Defra’s Secretary of State recently said that the highest standards of welfare would be a “unique selling point" for the UK, there remains considerable confusion. A 2017 parliamentary briefing perhaps describes it best:

> *"The terms of the Brexit negotiations will go a long way towards determining what animal welfare protections are adopted, amended or discarded. This may lead to the same, stronger or weaker regulations than those currently agreed. The Prime Minister has recently ruled out the possibility that the UK would remain a member of the Single Market, meaning EU legislation will cease to have effect after the UK formally leaves the EU. But considerable uncertainty still remains. So while nearly everyone believes Brexit offers an opportunity to change the system, no one can agree precisely how." (UK Parliament, 2017)*

1.2 Public perception of broilers

A recent increase in awareness of farmed poultry has almost exclusively been directed towards laying hens, with largescale campaigns to end battery cage systems spreading internationally. The imagery of caged hens proved successful in causing public outrage and an EU directive (Council Directive 1999/74/EC) was issued that no new cage systems could be built after 2003, and any existing cages should be replaced with furnished cages or an alternative system by 2012. In the UK, there was a sustained increase in demand for free range eggs, with the percentage of eggs coming from free range systems increasing from 10% in 1996 to 48% in 2016 (Defra, 2017). Comparatively, there is widespread misunderstanding of the source of chicken meat and the welfare concerns in broiler chicken production. An EU report (EU Commission, 2000) on the welfare of broilers confirmed that there was less consumer sensitivity to broiler welfare, due to at least three main reasons: 1) they lacked a clear "symbol" of mistreatment (such as cages), 2) welfare scientists are only able to suggest relative improvements to their welfare, for example reducing growth rate, rather than provide a binary "with/without" solution, and 3) there is a general misunderstanding about what a broiler is and what the issues are, for example many people believe broilers are kept in cages.

Research has frequently reported that consumers have very little knowledge of what broilers are or how they are raised, and experience shock when presented with the reality of intensive broiler housing (Köhler, 1999; Hall and Sandilands, 2007). Demand for clearer labelling systems have been made from research scientists (e.g. de Jonge and van Trijp, 2013; Heerwagen et al., 2015), the Farm Animal Welfare Committee (FAWC, 2006) and EU reports (EU Commission, 2009), however confusing labelling systems remain a problem and are likely to limit our understanding of consumer demand for higher welfare. There is a willingness to pay more for high welfare products (Mayfield et al., 2007), however retailers and supermarkets use animal welfare as a method of differentiating their product, and as such they have a substantial amount of control over the standards that suppliers adhere to. Through advertising, promotions and shelf space for high welfare products, they can heavily influence consumer purchasing behaviour and production practices (Vanhonacker and Verbeke, 2014).

1.3 Broiler chickens

In many ways, broiler chicken production is a triumph of modern science, allowing a luxury item to be transformed into a staple of modern diets (Clarke, 2014). Historically, chicken flock sizes were small and birds would lay eggs for several years before being killed for meat. An increase in demand for cheap food and the end of feed rationing after WWII revived the UK's struggling poultry sector, and imports of specialised "broiler" or "grilling" chickens from America in the 1950s saw a divergence begin between egg layers and broiler chickens. For broiler chickens, there was a focus on meat yield, meat quality, growth rate and feed conversion efficiency. In the 1960s, a broiler chicken would reach its slaughter weight of 2kg at 63 days with a feed conversion ratio of 2.5. Modern broilers now reach 2kg in around 34 days, with a feed conversion ratio of 1.5 (Aviagen, 2014).

Broiler housing has also changed dramatically, with technological advances paving the way for an increase in intensification. Flock sizes are now in the tens of thousands, with widely used automated systems that control lighting, temperature and humidity. Broiler sheds are typically large metal-framed structures with concrete floors and walls, and several feeder and drinker lines that supply food and water *ad* *libitum*. Woodshavings, or another form of litter, are provided in a moderately deep bed from day 0 and not changed throughout the production cycle. Advanced biosecurity measures and "all in, all out" production systems have controlled the risk of disease, and monitoring systems are in place in slaughter houses to detect heightened damage to carcasses which can indicate housing or transport issues (Haslam et al., 2008; Cox and Pavic, 2010). This increase in efficiency and intensification has led to an exponential increase in the number of broilers raised in the UK and globally. There are over 828 million broilers slaughtered in the UK alone, a figure that has nearly doubled since the 1990s (Defra, 2017). In 2014, there were a staggering 62 billion broilers slaughtered worldwide (FAOstat, 2014).

Although some would argue that there are benefits to intensive systems, this method of broiler production has come at a clear cost to bird welfare (Bessei, 2006). Broilers early growth rate and high body weight are directly linked to a susceptibility for metabolic and skeletal disorders, and birds show a marked and abnormal reduction in locomotor behaviour. Contact dermatitis is prevalent in houses with poor litter quality, and management measures such as high stocking densities and lighting regimes have been criticised for their effect on bird welfare. The housing itself also offers little stimulation to broilers which is likely to compound their low activity levels and cause boredom and frustration (Newberry, 1995, 1999; Bessei, 2006). Although broiler welfare is becoming a more common research topic, there remains a large body of work focusing on laying hen welfare, which is often difficult to extrapolate to broilers due to their substantial morphological and behavioural differences.

1.4 Broiler welfare concerns

Animal welfare is a word that came from society rather than from science (Duncan, 2005), and debate still exists on its most appropriate scientific definition. The common meaning of animal welfare is that it concerns the physical and mental well-being of an animal, with those two criteria being assigned varying importance (Duncan and Petherick, 1991; Dawkins, 2004; Duncan, 2005). The 2007 EU Directive, which came into force in 2010, is the most recent legislation passed for the protection of chickens reared for meat (Council Directive 2007/43/EC). The directive largely governs aspects of the birds environment, such as lighting, stocking density and litter requirements. Management practices, such as twice daily house inspections and personnel training, were also included. No additional UK legislation has been passed concerning broiler welfare, however welfare assurance schemes (e.g. RSPCA, 2017b) and certain retailers (e.g. M&S, 2015) have additional requirements.

1.4.1 Lameness

Leg weakness in broilers has been described for several decades as a problem associated with selection for high productivity traits (Mercer and Hill, 1984). However, the issue remains relatively widespread. Despite alterations to commercial breeding programmes that have improved the incidence of lameness (the disabling form of leg weakness) over the past 20 years (Kapell et al., 2012), these advances are likely to be limited by the link between leg health, body weight and growth rate (Bessei, 2006). A 2008 survey (Knowles et al., 2008) of over 50% of UK broiler flocks found that 98% of broilers had some detectable gait abnormality by the time they reached slaughter weight, and 28% had a gait score of 3 or above which indicates lameness (on a scale of 0-5, where 0 is completely normal and 5 is unable to stand; Kestin et al., 1992). A 2013 investigation of intensive broiler houses in the UK, the Netherlands, France and Italy found an average of 15.6% of birds with gait scores of ≥3, with a wide variation between flocks (5 and 95% quartile: 0.5 and 52% respectively; Bassler et al., 2013). More recent studies of Norwegian broiler flocks have found the average birds with gait scores of ≥3 to be 25% (Kittelson et al., 2017) and 19% (Vasdal et al., 2018). However, smaller trials do report significantly lower average gait scores (e.g. Silvera et al., 2017), which is likely to be due to large variation between farms and the subjective nature of gait scoring (Dawkins et al., 2004; EU Commission, 2000).

Leg disorders are considered to be a major cause of poor welfare in modern broilers (EU Commission, 2000), compromising both bird health and mental well-being. Lame birds show a reduction in walking, standing and performing behaviours while upright (Weeks et al. 2000). Extremely lame birds are likely to have difficulty reaching feeders and drinkers, which are increasingly raised over the production cycle (Butterworth et al., 2002), resulting in poor performance or risk of starvation and dehydration. Lameness is also assumed to be a painful condition. Broilers possess the necessary nociceptors and behavioural responses to noxious stimuli that indicate their ability to feel and experience pain (Gentle and Hill, 1987; Gentle, 2011). Broilers will also show an improvement in walking ability and speed when given analgesics (McGeown et al., 1999, Caplen et al., 2013), which is a common method of indirectly measuring an animal's pain status. However, studies investigating whether lameness is painful for broilers differ significantly in their analgesic strategies, methodology and outcome measures, which makes conclusions difficult. Danbury et al. (2000) found that lame broilers will self-select feed containing analgesics more than their healthy counterparts and show improved walking ability. However, with a substantially different methodology that did not involve training birds to differentiate feed by colour, Siegel et al. (2011) found no effect of lameness on dosed feed intake.

There is also a wide variation in dosing levels in analgesic studies. For example, while birds given 4 mg/kg of the NSAID carprofen showed no improvement in gait (Corr et al., 2007), others given 25 mg/kg showed an increase in motility, albeit with observable instability (Caplen et al., 2013). Increasing the dosage again to 35 mg/kg did not allow increased walking speed, probably because of the previously noted instability, but birds were able to stand for longer in a water bath (Hothersall et al. 2016). Similarly, while one trial found that lame broilers were able to complete an obstacle course faster once they had been injected with 2 mg/kg^{-1} of the opiod butorphanol (Singh et al. 2017), another found that doubling the dosage to 4 mg/kg^{-1} of butorphanol had a soporific effect and increased the time it took a lame bird to complete an obstacle course (Hothersall et al. 2016).

Although this research is as yet inconclusive, lame broilers do show considerable behavioural changes and gait adjustments. It is likely that future research will clarify our understanding of lameness induced pain in broilers.

1.4.1.1 Causes of lameness

The causes of broiler lameness can generally be placed into three non-mutually exclusive categories: infectious, developmental and degenerative (Bradshaw et al., 2002). Infectious causes are thought to be the most common cause of lameness, with skeletal deformities accounting for the majority of remaining cases (Butterworth, 1999). Infectious causes include bacterial chondronecrosis with osteomyelitis, synovitis, and infectious stunting (EU Commission, 2000; Bradshaw et al., 2002). The main developmental disorders are characterised by angular deformities in the long bones, usually caused by improper bone or cartilage formation, including valgus/varus deformities and dyschondroplasia. These deformities can affect broiler walking ability and cause secondary soft tissue pathologies (EU Commission, 2000). Degenerative disorders are usually more apparent in older birds, and include osteochondrosis (usually tibial dyschondroplasia), degenerative joint disease, spontaneous rupture of gastrocnemius tendons, and epiphyseolysis of the femoral head (Bradshaw et al., 2002).

Bacterial chondronecrosis (BCO) is the most common form of infectious lameness, with reports suggesting that over 1% of birds raised in conventional systems will be affected (Wideman, 2015), which would have equated to around 8.3 million birds in the UK in 2016. Broilers high growth rate and rapid increase in body weight puts mechanical stress on their immature skeletons which can cause microfractures that are colonised by opportunistic bacteria, usually *S. aureus* (Butterworth, 1999; Wideman, 2015). Inactivity and long periods of sitting may also interfere with blood flow and prevent proper cartilage development, increasing the risk of BCO (Wideman, 2015). Inaccessible for the bird's immune system, this infection leads to abscess formation and necrosis of cartilage and bone tissue (Butterworth, 1999). Birds will rapidly become lame and typically die within 2-5 days of outward signs of infection, which can include using wings for support when moving, vocalisations if joints are manipulated, a weak response to external stimuli, and sharp reduction in feed and water intake (McNamee and Smyth, 2000). Bacterial and viral agents can also cause synovitis (arthritis), which is inflammation and swelling of joints, and infectious stunting which prevents proper nutrient absorption leading to stunted growth and malnutrition (EU Commission, 2000).

Developmental disorders in broilers can largely be attributed to some combination of their growth rate, genetics, conformation, inactivity, nutrition and intensive management practices (Bradshaw et al., 2002). Varus/valgus deformities and tibial dyschondroplasia are the most prevalent developmental disorders (EU Commission, 2000). Varus and valgus deformities are characterised by inwards or outwards angulation of the lower part of the leg (Julian, 1984), while tibial dyschondroplasia occurs when there is an abnormal build-up of uncalcified chondrocytes in the growth plate, resulting in improper bone formation. These lesions can lead to either a fracture in the growth plate or the development of an abnormal tibial plateau angle, resulting in varus or valgus deformities (EU Commission, 2000; Bradshaw et al., 2002). A strong correlation between increased growth rate and varus/valgus deformities has been found in several studies (Mercer and Hill, 1984; Akbas et al., 2009; Shim et al., 2012) although the literature is inconsistent (Le Bihan-Duval et al., 1996; Bradshaw et al., 2002; Rekaya et al., 2013). Similarly, tibial dyschondroplasia can be increased with genetic selection (Yalçin et al., 2000) and strong genetic connections with growth rate have been reported (Sorensen, 1992; Bradshaw et al., 2002). Kestin et al. (1992) managed to almost eliminate leg problems in broilers by random breeding, despite housing the birds using typical intensive management practices.

However, genetic factors do not appear to be solely responsible for the development of leg problems in broilers. Weak correlations between bone quality and leg disorders (González-Cerón et al., 2015a), and reports of low to moderate heritability for these conditions (e.g. Rekaya et al., 2013; González-Cerón et al., 2015b) points to the impact of environmental factors. Shorter dark periods (Bassler et al., 2013), litter moisture (Dawkins et al., 2004), early hatching (Groves and Muir, 2017), and nutrition (Waldenstedt, 2006) have all been linked with the development of non-infectious leg disorders. Forcing birds to exercise also has a positive effect on leg health (Thorp and Duff, 1988; Reiter and Bessei, 1995) and there is potential for environmental enrichment to improve tibial dyschondroplasia (Kaukonen et al., 2017a), bone quality and walking ability (Bizeray et al., 2002a). Abnormal gaits and lameness can also occur in broilers with no obvious disorder or injury (Julian, 1998). Broilers have been selected for increased breast muscle, or pectoral hypertrophy, which now equates to 18% of their body mass, compared to 9% in a less selected heritage line (Schmidt et al., 2009). This has displaced their centre of gravity forwards and made birds unstable, leading to compensatory gait modifications. Broilers have a wide stance, a short stride length and exaggerated lateral motions, resulting in a stereotypical waddle (Reiter and Bessei, 1997; Corr et al., 2003; Caplen et al., 2012). It can therefore be difficult to determine whether a broiler's gait is primarily influenced by its morphology, by discomfort and pain, or by both.

1.4.1.2 Measuring lameness

Gait scoring is frequently used to assess broiler walking ability, benefiting from being inexpensive and practical. In the commonly used Bristol gait score (Kestin et al., 1992), broilers are scored on a scale of 0 to 5, where 0 indicates a normal gait with no detectible abnormality and a score of 5 is given when a bird cannot stand. Kestin et al. (1999) suggested that birds with a gait score of 3 and above should be considered likely to suffer from chronic pain or discomfort associated with their immobility, an approach which has been widely adopted. The relationship between gait score and underlying pathology is not well understood (Bradshaw et al., 2002). Lame birds with a gait score of 3 and above were found to have a number of pathologies not seen in sound birds, particularly bacterial chondronecrosis with osteomyelitis (McNamee et al., 1998; Butterworth et al., 2001). While some studies have found an association between lameness and tibial dyschondroplasia (Vestergaard and Sanotra, 1999), others have not (Garner et al., 2002). However, broilers with a high gait score but no apparent pain inducing pathology may still suffer from an inability to reach feeders and drinkers and perform normal behaviours (Bradshaw et al., 2002).

Although gait scoring can be easily applied on-farm and requires little equipment and training, it has been criticised for its subjectivity and lack of inter-rater reliability (EU Commission, 2000). Efforts to improve the Bristol method have been made both by increasing the level of detail (Garner et al., 2002) and by collapsing categories to simplify the scale (Webster et al., 2008). The Modified Gait Scoring Method was developed in an attempt to reduce error between studies (Garner et al., 2002). This gait score retains the 0-5 scale but includes more specific detail, including time limits for recognising abnormalities, and has slightly higher test-retest and inter-rater reliability than the Bristol gait scoring method (Garner et al. 2002). An alternative to gait scoring, "latency to lie", has also been developed and can be applied practically on farms (Weeks et al., 2002). Latency to lie involves placing broilers in a shallow bath of water and measuring the time it takes them to sit, producing a more objective measure of leg health. The assumption is that water is aversive to broilers and those capable of holding their body weight will stay standing for longer, with birds experiencing pain or unable to stay upright sitting down faster. There was a strong inverse relationship between gait score and latency to lie, and it may be a more sensitive test of the bird's experience of lameness.

Advances in automated ways of measuring walking ability have removed the problem of inter-related reliability, however they are largely restricted to providing detailed information on the walking patterns of birds in a laboratory setting. Broiler gait analysis has been performed using video tracking, pressure plates and infra-red motion detection (Corr et al., 2007; Caplen et al., 2012). Links have also been found between gait score and automated video analysis of lying bouts and latency to lie (Aydin et al., 2015). This technology requires the separation and classification of individual birds, which makes it unusable in a commercial environment. However, techniques that allow for "crowd" analysis such as optical flow (Dawkins et al., 2009) and flock movement away from approaching humans (Silvera et al., 2017), have more potential for on-farm use.

1.4.2 Contact Dermatitis

Extended periods of contact with poor quality litter can cause various types of contact dermatitis in poultry, affecting the feet (footpad or podo- dermatitis), hocks (hock burn) and breast (breast burn). The high litter moisture, and chemical burning effect of ammonia from urea, can damage the skin and cause ulceration and lesions (Dawkins et al., 2004; Haslam et al., 2007). Leaks from drinker lines can also rapidly worsen litter quality in the house, and wet litter alone without excreta has been shown to cause fully developed lesions (Mayne et al., 2007). A 2007 survey (Haslam et al., 2007) of 206 UK flocks, raised through 4 major UK broiler companies, found the prevalence of breast burn to be generally low, with an average of 0.002% (ranging from 0 to 0.12%), and hock burn prevalence to range from 0 to 33%, with an average of 1.29%. Footpad dermatitis was the most common condition, with an average of 11%, ranging from 0 to 72%. The extent of the problem was highlighted in a recent study of 53 UK flocks (on one farm; Dawkins et al., 2017), which found the prevalence of footpad dermatitis to be 51.6% (SD 23.4) and of hockburn to be 20.5% (SD 16.4). These lesions are assumed to be painful depending on their severity (Gentle et al., 2001; Gentle, 2011), contribute to bird lameness, and represent a significant reduction in bird welfare and production (Martland, 1985; de Jong et al., 2014).

1.4.3 Inactivity

The relationship between inactivity and broiler leg health appears to be circular, with low activity contributing to leg weakness, and painful leg disorders in turn reducing locomotion. Lame broilers will spend up to 86% of their time sitting down by slaughter weight, limited by their disability and the pain of moving (Weeks et al., 2000). However, broilers with no obvious signs of lameness will still spend 76% of their time sitting down by 6 weeks of age (Weeks et al., 2000). This is an extreme departure from their red junglefowl ancestors who spend the majority of their time performing active behaviours and foraging (Dawkins, 1989; Schütz and Jensen, 2001). Selection for rapid growth rates and high body weight are both associated with a reduction in active behaviours and increase in leg disorders (Bizeray et al., 2000; Kestin et al., 2001). Young broilers given an artificially high body weight showed defective long bone formation after 4 days (Reich et al., 2005). It is also likely to require more energy to move when birds are heavier; older broilers have larger thigh muscles and feet which implies that the energetic cost of swinging their legs increases (Paxton et al., 2014). Broilers will walk further when part of their body weight is alleviated using harnesses, and artificially placing more weight on slow growing broilers leads to a reduction in locomotion (Rutten et al., 2002; Đukić-Stojčić and Bessei, 2011).

In humans, hypoactivity prevents proper development of the musculoskeletal system, reduces bone mass and is a risk factor for osteoporosis (Rittweger et al., 2005; Pinheiro et al., 2009). The same is ostensibly true for chickens. Caged broilers showed significantly reduced bone mass and quality compared to those able to move around (Aguado et al., 2015), and it is thought that prolonged periods of sitting can cause an interruption of blood flow to vascularised bones and joints, preventing normal development and maturation (Wideman, 2015). When young broilers are forced to exercise, there is a reduction in leg disorders when they are older (Reiter and Bessei, 1995). Making broilers walk further to reach feeders and drinkers (Bizeray et al., 2002a,b), giving broilers space to range (Fanatico et al., 2005; Stadig et al., 2017) and providing perches (Tablante et al., 2003; Ventura et al., 2010) has had some mixed success in improving leg condition. Although broilers appear to be capable of moving further than they would choose to, suggesting an additional motivational component (Reiter and Bessei, 1994, cited by Bessei, 2006; Reiter and Bessei, 1995), encouraging locomotion in commercial housing has proved difficult.

1.4.3.1 Motivation

A lack of motivation to move around and forage may further explain the dramatic lack of activity seen in even very young broilers (Bizeray et al., 2000). Foraging in domestic fowl is an example of contrafreeloading, which describes a feeding strategy whereby animals will choose to work for food even though a source of identical food is freely available (Osborne, 1977). For example, rats will continue to press a lever that delivers a food pellet even when a bowl of “free” food pellets is placed in their enclosure (Jensen, 1963). In natural conditions, this strategy is believed to be an adaptive means of allowing animals to gather information about their environment, with this expenditure in energy being offset by the benefit of identifying novel food sources (Inglis et al., 1997). Although this adaptive behaviour remains present in domesticated animals, there appears to be a negative relationship between contrafreeloading and selection for high production traits. When red junglefowl are offered either an easy box of chicken feed, or a box of feed mixed with woodshavings, they will consume approximately 33% from the easy box and 67% from the feed mixed with woodshavings (Schütz and Jensen, 2001); a choice which requires them to search, scratch and separate the food rather than simply eating. However, in the same situation, laying hens will choose to obtain 15% of their food from the mixed box, while broilers will eat only 5% of their food from the mixed box (Lindqvist et al., 2006).

Broilers clearly will spend little effort exploring for food, an activity that occupies the majority of their ancestors’ time budget. In one study of broiler behaviour, foraging was only observed in 3% of observations, compared to 90% in red junglefowl (Dawkins, 1989; Weeks et al., 2000). This departure from normal behaviour patterns can be, in part, explained by broilers morphology and susceptibility to painful skeletal conditions (Bessei, 2006). However, the resource allocation theory offers a further motivational explanation (Beilharz et al., 1993; Schütz and Jensen, 2001). This theory suggests that animals have a limited amount of resources that are allocated to different energy consuming life processes, for example reproduction, immune defence, food gathering etc. In selectively bred animals, energy is artificially reassigned to production traits. For broilers, energy that would have been allocated to extended periods of exploration has been redistributed to growth and muscle development. This absence of energy available for contrafreeloading may be responsible for broilers lack of motivation to move and contribute to their prolonged periods of sitting inactive. Evidence for this theory has been provided by studies that showed birds selected for poor feed conversion efficiency are more active than those selected for high feed conversion efficiency (Braastad and Katle, 1989; Schütz and Jensen, 2001). However, it is generally accepted that you cannot eliminate a behaviour through breeding, but rather you can increase the threshold before that behaviour will be performed (Hale, 1962 and Price, 1998; cited by Schütz and Jensen, 2001). Methods of bringing the threshold for exploration within reach may include reducing the energy required for broilers to move, reducing pathologies that cause moving to be an aversive painful experience, and providing a complex environment that stimulates birds to explore.

1.4.4 Fear

Fear is an adaptive behaviour system that has evolved as a means for animals to survive in dangerous environments (Misslin, 2003). As a response to potential or actual threats, particularly predation, animals display innate survival strategies. These include fight or flight, avoidance behaviours, tonic immobility, and submissive postures. However, while fear plays a vital role in animal survival, its persistence in domesticated animals that are largely protected from actual threats can be harmful. Domestic fowl are less fearful than their ancestors (Campler et al., 2009), as an intentional or unintentional consequence of domestication, and broilers show less vigorous fear responses than laying hens (Keer-Keer et al., 1996). However, broilers can still display extreme escape reactions, which can cause birds to pile on top of one another or run into obstacles, risking suffocation or serious injury (Mills and Faure, 1990; Jones, 1996). While serious injuries can cause chronic pain, milder injuries such as scratches and bruising can also increase the risk of infection, and increase the incidence of carcass downgrading at slaughter. Increased fearfulness in broilers has also been associated with a reduction in feed conversion efficiency, productivity, growth rate, and immune response, and an increase in mortality in young broilers (Hemsworth et al., 1994; Jones, 1996; Zulkifli et al., 2002; Wang et al., 2013). In addition to a risk to health and productivity, fear is also considered a state of suffering in animals. Rather than simply responding to a stimulus with a reflex, birds appear to experience fear as a negative emotional state. Duncan and Filshie (1980) exposed chickens to a rapidly expanding balloon which startled them and caused escape reactions, with birds running away into another nearby chamber. The balloon expanding was then paired with a warning light and birds quickly learned to expect the balloon to expand and would move to the alternate chamber once the warning light was shown. This suggests that chickens had an unpleasant mental experience and would try to avoid being frightened (Duncan and Petherick, 1991).

1.4.4.1 Measuring fearfulness

Measuring avoidance behaviours or “flightiness” in poultry is a practical method of assessing fearfulness in commercial conditions. An observer approaches birds and measures how many remain within a set distance (Jones, 1993), or at what distance the birds withdraw from the observer (flight distance; Graml et al., 2008). Generally, birds that move further away from the observer or have longer flight distances are considered to be more fearful than those that show less avoidance of a human. However, the vast majority of studies validating these avoidance tests have used laying hens. Poor leg health in broiler chickens could hinder their ability to avoid an observer and influence the validity of these measures. A recent study found that broilers with high gait scores showed less withdrawal behaviour (Vasdal et al., 2018), suggesting that fear tests involving broilers walking ability may not be appropriate. Comparing the duration of tonic immobility in birds placed on their back or side can also be used to test fearfulness. Tonic immobility is an innate behaviour that can be stimulated with brief manual restraint. Birds that are held down by an observer will remain immobile for a period of time, showing a reduced responsiveness to external stimuli and a temporary suppression of the righting response (Jones, 1986). Longer periods of tonic immobility are associated with increased fearfulness (Jones, 1986; Jones et al., 1988), however there also appears to be a relationship between leg disorders and tonic immobility (Vestergaard and Sanotra, 1999). Latency to approach a novel object or to explore a novel area may also indicate the level of fear that birds are experiencing (Jones, 1996). Inhibited, inactive, quiet birds are considered to be more fearful than those that investigate any novel aspects, explore, vocalise and eat (Jones, 1989; Jones, 1996). The interpretation of these tests can be difficult and they are more practical in laboratory conditions (Forkman et al., 2007).

1.4.4.2 Modifying fearfulness

Modifying fearfulness in broilers is important both to relieve the underlying negative emotional state, and to avoid injury from overreaction to sudden stimuli. Rough handling can increase the duration of tonic immobility in young broilers, which indicates the importance of positive human-animal relationships in reducing fearfulness (Jones, 1992). Regular gentle handling has been shown to reduce fear responses in several studies (Jones and Faure, 1981; Jones, 1992; Jones, 1994). Traveling is also considered to be a major stressor for broilers, with the length of the journey positively associated with duration of tonic immobility (Cashman et al., 1989). Within commercial housing, the lack of protective cover available may be frightening. The ancestors of domestic fowl would have relied heavily on vegetative cover for shelter and protection from predation. Chickens appear to be attracted to protective cover and will perform more vulnerable behaviours, such as preening and resting, in the presence of cover panels (Newberry and Shackleton, 1997; Cornetto and Estevez, 2001b). The provision of environmental enrichment in the home pens of domestic fowl has been successful in improving a variety of fear measures, including increased vocalisations in novel object tests, shorter latency to approach a novel object, and reduced avoidance of humans (Jones and Waddington, 1992). However, it is often difficult to extrapolate laboratory results to commercial settings, and recent research using enrichments in commercial broiler housing found no positive effect on novel object exploration or avoidance of an observer (Bailie and O'Connell, 2015).

1.5 Dustbathing

Dustbathing is a conspicuous activity in birds, comprised of seated kicking and shuffling motions that transfer dust into their raised feathers. The purpose of dustbathing is likely to be to remove ectoparasites and maintain feather condition, which gives it significant adaptive value (van Liere and Bokma, 1987; Martin and Mullens, 2012). Dustbathing is seen in many species and has been extensively studied in domestic fowl (Olsson and Keeling, 2005). Patterns of dustbathing can differ between and within individuals, however the basic structure of a dustbathing bout is as follows (van Liere, 1991):

- A bout will typically begin with a standing bird scratching and bill raking at the dustbathing substrate, before fluffing its feathers erect and squatting down.

- While sitting, the bird will shift dust in amongst its feathers using vertical wingshakes, head rubbing and prone kicking motions with one leg. For vertical wingshaking, the bird will scratch dust backwards and upwards with both legs, and then shuffle its wings to throw dust in between its feathers. The bird may also rub its head over the dust and lie prone on one side, kicking dust over itself. Bill raking the substrate closer to its body usually precedes and ends this pattern of behaviours.

- After several of the above sequence, the feathers are flattened and the bird will lie on its side, stretching out its leg and rubbing itself against the substrate. This side-lying and side-rubbing can be interrupted by vertical wingshakes, head rubbing and prone kicking. This phase can be mistakenly interpreted as resting.

- At the end of the dustbathing bout, which lasts approximately 20 minutes in laying hens after the initial vertical wingshake, the bird will stand and perform a bodyshake which removes excess dust from the feathers.

In junglefowl, bill-raking is first seen at around 2 days of age, and the remaining elements of dustbathing continue to appear until it is presented as a complete activity at day 10-12 (Kruijt, 1964). Low levels of dustbathing are observed during the first week, increasing to 2-3 times a day in weeks 2-3, before the behaviour stabilises to once every 2 days after week 4 (Kruijt, 1964; Hogan et al., 1991; Hogan and Van Boxel, 1993). Dustbathing follows a diurnal pattern, with a peak in dustbathing in the middle of the day clearly seen in adult birds (Hogan and Van Boxel, 1993). Domestication does not seem to have had a significant impact on dustbathing behaviour, with similar overall frequencies and patterns of dustbathing seen in both junglefowl and modern laying hens (Vestergaard et al., 1990; Schütz and Jensen, 2001).

Research largely focusing on laying hens has shown that a complex interaction of internal and external factors control the performance of dustbathing. When birds are prevented from dustbathing, they compensate by performing additional dustbathing at their next opportunity, suggesting a build-up of internal motivation (Hughes and Duncan, 1988; Vestergaard, 1982; Vestergaard et al., 1999). The complete components of dustbathing and the diurnal pattern will develop in the absence of any dust, and birds will perform the elements of dustbathing on wire if no suitable substrate is offered (Vestergaard et al., 1990; Petherick et al., 1995; Vestergaard et al., 1997). This dustbathing behaviour in the absence of a substrate is considered a "vacuum" behaviour by those following Lorenzian thinking (Vestergaard et al., 1999) and "sham" dustbathing by those arguing that birds are trying to use feed as a dustbathing substrate (Lindberg and Nicol, 1997; Olsson and Keeling, 2002a; Moroki and Tanaka, 2016). Feather condition does not appear to be a significant control factor of dustbathing; chickens without feathers, without oil glands, and with only visual but not physical access to dust will all still dustbathe (Nørgaard-Nielsen and Vestergaard; 1981; Vestergaard et al., 1999). Dustbathing can also be stimulated by environmental changes. Increased dustbathing is seen when birds are in sight of a dustbathing substrate, housed under brighter light intensities and higher environmental temperature, and in the presence of other dustbathing birds (Petherick et al., 1995; Duncan et al., 1998).

When a behaviour is controlled by internal factors, and reducing motivation is achieved through performance, continually preventing this behaviour is likely to cause a build-up of unsatisfied motivation leading to frustration and stress (Mason and Burn, 2011). The stress that accompanies thwarted dustbathing was demonstrated by Vestergaard et al. (1997), who found elevated plasma corticosterone levels in hens raised on sand and then transferred to wire. This physiological indicator for stress was not seen in birds raised on wire and then transferred to sand, instead these birds showed a reduction in stereotypical pecking and a substantial increase in dustbathing. A vocalisation linked to frustration, the gakel-call, is also recorded when birds are trained to expect access to a dust bath and are then obstructed (Zimmerman et al., 2000). Birds will push through a weighted door to get access to peat which they then dustbathe in (de Jong et al., 2007), although there is mixed evidence that birds are willing to “pay a price” for access to a dustbathing substrate (Widowski and Duncan, 2000). A recent study found that the anticipatory behaviour displayed by laying hens was greater when they were expecting a dustbathing substrate compared to a food reward (McGrath et al., 2016). Birds had been deprived of a dustbathing substrate continually but only feed restricted for two hours prior to the test, which may explain why they ranked access to a dusty substrate above access to food. This is supported by Dawkins (1983), who found that chickens would choose access to food over litter when they were feed deprived, but overwhelmingly chose litter if they were not hungry.

1.5.1 Dustbathing in broilers

Despite dustbathing appearing to be an important behaviour for domestic fowl, there is a general consensus that it makes up very little of broilers time budget. In a 1988 study on the time budgeting of commercial broilers, no dustbathing was observed at all, leading the authors to conclude that dustbathing may not be an important behaviour to broilers (Murphy and Preston, 1988). However, they only collected data on 19 broilers for 1 hour on each observation day, between days 27 to 50. Later studies of broilers in laboratory conditions have reported the proportion of birds observed dustbathing on woodshavings to be 0.34% (Weeks et al., 2000), 0.20% (Kristensen et al., 2007), < 1% (Alvino et al., 2009) and 0.48% (Schwean-Lardner et al., 2012a). Although information on dustbathing at a commercial level is limited, recent on-farm studies reported observations of dustbathing to be 0.3-0.46% (Bergmann et al., 2017) and 0.18% (Bailie et al., 2013). However, when broilers were kept in cages and given access to sand for one hour a day, 20 out of 47 birds dustbathed every single day between days 19 and 40, and the rest dustbathed an average of every 2.5 days (Stub and Vestergaard, 2001). Similarly, Vestergaard and Sanotra (1999) found that the majority of caged broilers without leg issues would dustbathe almost every day when given the opportunity, and displayed a rebound effect when deprived of dust. Even broilers with dyschondroplasia dustbathed after a three-day period of deprivation (Vestergaard and Sanotra, 1999). The authors conclude that low levels of dustbathing seen in many broiler studies are likely to be due to poor leg health and wet litter, rather than a reduced motivation to perform dustbathing. This suggests that, given the opportunity, broilers are still highly motivated to dustbathe and could therefore experience stress if thwarted. It is also worth noting that our perspectives of time budgets in broiler chickens are likely to be skewed; although the instance of 0-1% dustbathing could allow it to be interpreted as a largely irrelevant behaviour to broilers, the amount of time broilers spend feeding is only 5-13% (Weeks et al., 2000; Schwean-Lardner et al., 2012a; Deep et al., 2012; Bergmann et al., 2017), which does not reflect a low importance of feeding to broilers.

There are several reasons why abnormally low dustbathing may be being observed in broilers, including sampling techniques, physical limitations and environmental conditions. Scan sampling allows numerous animals to be observed simultaneously, which is useful in assessing group behaviour, and is frequently employed for behavioural studies. However, as dustbathing contains several elements that are similar to rest and pecking behaviours, it is likely that this technique underestimates the amount of dustbathing if observers are not specifically looking for dustbathing. This may be especially difficult in trials using woodshavings, as the litter will not be as visible in their feathers as other substrates, such as peat. Experiment practicalities may also reduce the number of dustbathing bouts observed if, for example, observations are consistently taken outside of peak dustbathing periods (Vestergaard et al., 1990).

It is well understood that broilers show a significant reduction in activity, and spend the majority of their time performing sitting or resting behaviours (e.g. Weeks et al., 2000). As dustbathing is an active behaviour that requires energy, a reduction in dustbathing is likely in modern broilers, especially as birds become heavier and it requires more energy to move. Despite this, a reduction in dustbathing with age is rarely reported (e.g. Weeks et al., 2000; Shields et al., 2004; Shields et al., 2005). In fact, several studies demonstrate an increase in dustbathing over the production cycle (Weeks et al., 1994; Bokkers and Koene, 2003; Bergmann et al., 2017). This suggests that broilers motivation to dustbathe remains high. As discussed, energy expensive behaviours that are not ‘adaptive’ for broilers, such as contrafreeloading, are much reduced. However, dustbathing does not seem to be as affected by domestication parameters as foraging; junglefowl and laying hens for example showed similar levels and patterns of dustbathing (Vestergaard et al., 1990; Schütz and Jensen, 2001). The high incidences of painful leg disorders seen in broilers are also likely to reduce levels of dustbathing, although the literature is sparse and inconsistent. Weeks et al. (2000) found no difference in dustbathing in broilers with gait scores of 0, 1, 2 or 3. However broilers with dyschondroplasia have been shown to dustbathe significantly less than their sound counterparts (Vestergaard and Sanotra, 1999).

Commercial conditions are also likely to inhibit dustbathing. Laying hens show a difference in dustbathing between natural and commercial conditions, with dustbathing bouts frequently being shorter and more likely to be interrupted in both caged systems and aviaries (Louton et al., 2016). Domestic fowl show reduced dustbathing when raised with a short dark period (23L:1D; Schwean-Lardner et al., 2012a), wet litter (Moesta et al., 2008), and low light intensities (Duncan et al., 1998; Kristensen et al., 2007), all of which can be standard conditions in commercial broiler housing. Broiler litter is likely to become unsuitable for dustbathing once it becomes wet and compacted, effectively causing a period of deprivation. When laying hens are raised on litter and then transferred to wire, or offered sand and then returned to woodshavings, they will stop dustbathing for an extended period of time (van Liere et al., 1990; van Liere and Wiepkema, 1992; Vestergaard et al., 1997). Even in good condition, woodshavings may be a suboptimal dustbathing substrate. Laying hens, for example, will push through a weighted door to dustbathe in peat but not in woodshavings (de Jong et al., 2007). It is therefore possible that any reduction in dustbathing in broilers is a symptom of deprivation rather than reduced motivation.

1.5.2 Substrate preferences

Generally, chickens appear to be more attracted to dustbathe in materials with fine particles, such as sand and peat, rather than materials with larger particles such as straw and woodshavings. Laying hens raised on woodshavings and then given access to sand, woodshavings, peat and sawdust performed significantly more dustbathing in peat than any other substrate, and dustbathing bouts were longer in peat (Petherick and Duncan, 1989). Broiler chickens will also explore and dustbathe in peripheral areas of their pen containing peat (Newberry, 1999). Hens show a willingness to push a weighted door to dustbathe in peat, suggesting a strength of demand that was not seen for sand, woodshavings and wire (de Jong et al., 2007). When comparing peat and sand, very little difference was found in the frequency of dustbathing bouts, suggesting they are similarly suitable (Duncan et al., 1998). A preference for sand over alternative substrates has also been reported. When broilers were offered either sand or straw, the number of days that no dustbathing was observed was doubled in broilers provided with straw (Vestergaard and Sanotra, 1999). When offered either sand, rice hulls, paper or woodshavings, broilers spent the most time and performed the highest number of vertical wingshakes in sand (Shields et al., 2004). Later studies similarly found a preference for dustbathing in sand over woodshavings, rice hulls, straw and recycled paper roll (Toghyani et al., 2010; Villagrá et al., 2014). Red junglefowl chicks further demonstrate a preference for dark sand over white sand (Vestergaard and Hogan, 1992).

The quality of dustbathing may also be affected by the available substrate. Hens housed in cages without any litter show fragmented sham dustbathing bouts (Appleby et al., 1993; Lindberg and Nicol, 1997). Dustbathing bouts performed in woodshavings are longer than those performed on sand (van Liere, 1991), which is suggested to reflect a lack of functional feedback from the woodshavings. The smaller particles in sand are more effective at penetrating the feathers, which birds learn can maintain short term feather condition (van Liere, 1991). Junglefowl will similarly dustbathe for longer on wire cage flooring compared to sand, and are more likely to end bouts performed in sand with a bodyshake to remove excess substrate, suggesting a similar lack of feedback available on wire flooring (Vestergaard et al., 1990). Pecking and ground scratching behaviours may also be influenced by substrate. Higher amounts of foraging were observed in peat and woodshavings compared to sand and sawdust (Petherick and Duncan, 1989), and sand was found to attract a consistently high level of foraging while levels of foraging declined in woodshavings over time (Shields et al., 2004). However, no clear preferences have been found between peat, sand and woodshavings in relation to the “cost” they will pay to access these substrates for foraging (de Jong et al., 2007).

Early experience of litter appears to influence later substrate choices. Chicks that learn to dustbathe on feathers still performed 52% of their dustbathing on feathers even when given access to sand (Vestergaard and Lisborg, 1993). When trained to dustbathe on feathers, straw and woodshavings, laying hens will continue to perform dustbathing on the familiar substrate initially, however a preference for sand will quickly develop following exposure, despite no previous experience of it (Sanotra et al., 1995). Hens placed in wire cages will show most sham dustbathing on the wire if they were raised in cages compared to those raised on peat, however when peat is placed below the cages of both wire-reared and peat-reared birds, they show identical amounts of dustbathing. This suggests that although birds’ perception of a dustbathing material can be affected by early experiences, chickens show an innate ability to recognise ‘dust’ and adult behaviour is largely influenced by the present substrate available (Nicol et al., 2001). For example, hens reared without any litter will use peat to dustbathe in their first experience of it, and wire-reared birds will thereafter push the same weight of door to get access to peat as peat-reared birds (Wichman and Keeling, 2008).

1.6 Play behaviour

For many years, play behaviour was considered too speculative and anthropomorphic to be suitable for scientific study, even Tinbergen claimed that play might never be able to be “satisfactorily defined objectively” (Tinbergen, 1963; Burghardt, 2005). Progress in play research suffered from a general suspicion of attributing emotion or awareness to animals, particularly “lower” animals such as rodents and birds (Burghardt, 2005). However, play research over the last century has attracted multidisciplinary interest and has proved important in furthering our understanding of animal behaviour (Bekoff, 1984). Several definitions of play have been proposed, the simplest of these is that play is any purposeless motor activity (Bekoff and Byers, 1981; Bekoff, 1984), although whether a behaviour is purposeless may depend on the “inventiveness of the observer” (Bekoff, 1984). Burghardt (2005) considers play to be a heterogenous category with similar characteristics but separate origins and functions, and has developed several criteria that may be used to identify play. Indeed, defining play has become more difficult in recent years due to the diverse animals studied and the species-specific nature of play. Špinka et al. (2001) has proposed that the basic underlying function of play is to train animals for the unexpected, by allowing them to rehearse unpredictable situations. Play has long been associated with a positive emotional state (Špinka, 2011) and, due to the shift in focus towards giving an animal a “life worth living”, play research is becoming more relevant to the farming industry.

Play can generally be grouped into three categories: locomotor play, social play and object play. While these versions of play are readily recognised in many mammals, there is also increasing evidence of play in birds (reviewed in Ficken, 1977 and Diamond and Bond, 2003). Play behaviours in poultry have not been defined or clearly investigated, and there is a reluctance to consider several behaviours that may fit within the discussed definitions as “play”. Duncan (1998) included frolicking and sparring as examples of play behaviour in domestic fowl, but highlighted the lack of information available on these behaviours. Mench (1988) also tentatively suggested that “sparring appears to possess a characteristic of mammalian play”. Included within a Welfare Quality report, Keeling and Zimmerman (2009) test the ability of “play-fighting, play-running, and play-running+wing-flapping” to act as welfare indicators for broilers. Nicol (2015) included a short paragraph summarising the collective knowledge available on play in poultry, in which frolicking and sparring were considered to be possible examples of play. However, due to their short duration, lack of innovation and apparent loss from the ethogram in older birds, it was concluded that it “would be difficult to argue that they provide an example of social play” (Nicol, 2015). As mentioned, the public’s perception of intensive farming remains poor, and the long-held Five Freedoms used to assess animal welfare have been criticised for their lack of focus on positive welfare (FAWC, 2009; McCulloch, 2013). In 2007, play behaviour was included as one of the top three most promising indicators for positive experiences in domestic animals (Boissy et al., 2007). It seems of value then to give the existence of play in poultry more thought.

1.6.1 Sparring

There is a broad consensus that mock-fighting exhibited in many species is an example of play (Aldis, 2013). Play-fighting is commonly described as a behaviour that “involves the use of the species-typical behaviour patterns of agonism, which are used in a non-serious manner. That is, their use does not lead to the functional consequences that are derived from their serious use” (Pellisa and Pellisa, 1998). Kruijt (1964; cited by Ficken, 1977) points out that the pattern of sparring seen in junglefowl, being an incomplete version of adult fighting and sometimes directed at inanimate objects (including feathers and their own tail), is similar to other behaviours described as play. However, he considered using the term “play” to be superfluous and irrelevant in determining the organisation of the behaviour. A similar reluctance to consider sparring in domestic fowl as play remains, with many authors opting to either refer to it as some variation of non-aggressive fighting (e.g. threat) or simply as a type of aggression. The differences between sparring and aggression are quite distinct in early descriptions of the behaviour. Guhl (1958) described sparring in a laying strain as two chicks “jumping up and down, as adults do when fighting, but the chicks failed to deliver any blows with their beaks. The behaviour waned readily and the partners pursued other activities”. This sparring behaviour was initially observed in week 2, well before avoidance behaviours developed in week 5 and fighting in week 6 (Guhl, 1958). Similarly, sparring was observed by Dawson and Siegel (1967) in very young chicks, before the behaviour peaked between weeks 4 and 5 and was eventually surpassed by aggressive behaviours, with sparring not observed after the ninth week.

Play is considered to be an "opportunity behaviour" that is quickly lost from the ethogram under challenging conditions (Fraser and Duncan, 1998; Špinka et al., 2001). One of these conditions is a reduction in food availability. The sensitivity of play to a reduction in feed intake has been demonstrated in calves (Krachun et al., 2010), rhesus monkeys (Loy, 1970), deer (Müller-Schwarze et al., 1982) and rats (Siviy and Panksepp, 1985). The same may hold true for sparring. Mench (1988) found that broilers on a skip-a-day feeding regime were more aggressive but performed less sparring behaviours than those fed ad libitum. Feed-restriction leading to aggression has been shown in layers (Duncan and Wood-Gush, 1971) and broiler breeders (Shea et al., 1990; Mench, 2002). In a recent study using broiler breeders aged 10-21 weeks old, the amount of aggressive pecking observed was significantly different between two types of restricted feeding, but the amount of sparring (called "threats" in this paper) was unaffected (Girard et al., 2017). Mench (1988) also reported that the frequency of sparring was not related to the frequency of later aggressive interactions, either in feed restricted or ad libitum broilers. This could suggest a basic difference between the two behaviours. It has been suggested that threats may function as a way to avoid injury and maintain the established social hierarchy (Rushen, 1982; Queiroz and Cromberg, 2006). As broilers are slaughtered as juveniles, there is no clear social hierarchy to maintain. Indeed, broilers may not even create a pecking order in large groups (Estevez et al., 1997). Rushen (1982) found that dominant and subordinate hens were equally likely to initiate a sparring bout, however dominant individuals were more likely to reciprocate. Additionally, a positive correlation between threats and aggression was found in broiler breeders, which suggests threats were not being used as an alternative to aggression once social order was formed (Girard et al., 2017).

There have been several recent examples in which identifying juvenile sparring as aggression may affect the interpretation of trials. In a study designed to test the effect of crowding and perch availability on aggression in broilers, broiler chicks between 2 – 6 weeks old were housed at three different stocking densities and with four different perch designs (Pettit-Riley et al., 2002). Aggression was measured as either “threats” which was defined as “an encounter in which a bird stands with neck erect, and feathers raised in front of a second bird, which usually has its head at a lower level”, or “other” which included all other forms of aggression including chase, fight, fight with peck, leap, peck and stand-off. While the latter behaviours were very infrequent and grouped together, there were significantly higher numbers of threats. The frequency of threats peaked at 3-4 weeks, which is in agreement with Dawson and Siegel’s (1967) description of sparring. Contrary to the authors expectations; 1) the highest number of threats were observed in the least crowded treatment, 2) there were significantly more threats in open areas of the pen compared with at the feeders, and 3) there was a tendency for more threats in the mixed angled perch treatment (which took up the least floor space) and control with no perches. In short, broilers appeared to be sparring more in areas where there was space available and not near the feeders where more aggressive interactions were predicted. The authors conclude that perches do not necessarily reduce aggression but that it depends on the type of perch, area of the pen, and type of aggression that is observed. Interpreted with “threats” as a type of play behaviour, this study may in fact provide evidence that space is a limiting factor to play in broilers.

Evidence for this can be drawn from several other studies on juvenile fowl. Ventura et al. (2012) tested a similar prediction that perches, either simple bar perches or complex perches with multiple arms, would reduce the amount of aggression compared to barren controls. They used broiler chicks aged 2-6 weeks, and housed them in pens with varying stocking densities. Using an identical ethogram as Pettit-Riley et al. (2002), based on Estevez et al. (1997), “aggression” consisted of chase, fight, leap, peck, stand-off and threat behaviours. In this trial, they found no effect of stocking density on aggression, however aggression was reduced in the simple bar perch treatment and almost eradicated in the complex perch treatment which left less open space. This reduction in aggression occurred despite an increase use of central areas of pens when perches were present. Provision of perches has also resulted in a physiological stress response in broilers (Heckert et al., 2002), which the authors attribute to the infrequently used perches causing a reduction in available floor space. This increase in stress response may also theoretically reduce play behaviours. In addition to finding more chasing and display (assumed to be sparring/threats) but

less aggressive head pecking in broilers stocked at a lower density, Andrews et al. (1997) also found a cumulative effect. When broilers had been stocked at a lower density in week 2, more chasing and display was evident in broilers housed at a lower stocking density in week 4, compared to birds that had initially been stocked at a higher density. This suggests there may be an additional effect of facilitating sparring behaviours in younger birds on their later behaviour.
There is an inherently difficult and long process involved in proving that any behaviour is play. Play-fighting can be difficult to separate from aggression, even in children (Smith et al., 2004; Graham and Burghardt, 2010). However, the hesitation to discuss pre-aggressive sparring within a context of play may have limited our understanding of its motivation, function and welfare associations.

1.6.2 Frolicking

While sparring resembles aggression, and could therefore be a more disputed example of play, there does not appear to be any clear explanation of the function of frolicking. Kruijt (1964) did suggest that frolicking may be triggered by an escape reaction to the bird's own tail, although this remains untested. Frolicking appears to have been originally described as "emotion dissociated fleeing movements" by Lorenz (in Nice, 1943; cited by Ficken, 1977). This term referred to a behaviour, noticed particularly in young birds, that resembled a bird fleeing a predator but without any apparent stimulus. Frolicking does indeed resemble an exaggerated escape reaction; birds engage in an apparently spontaneous and purposeless burst of running, with excess flapping and rapid direction changes. The behaviour tends to be short, with birds resuming other activities directly following a bout. Frolicking appears to be "contagious", in that once one bird frolics several others will also begin to frolic, although not necessarily in the same direction (Guhl, 1958; Dawson and Siegel, 1967).

Given the lack of information available on frolicking, it may be useful to employ Tinbergen's four ethological aims (Tinbergen, 1963), with an additional fifth aim added by Burghardt in the context of play (Burghardt, 2005), to discuss the existing and absent literature. These five ethological aims needed to describe a behaviour are: control (the internal or external factors that control a behaviour), adaptive function (the purpose of this behaviour in terms of improving group or individual fitness), development (the pattern of change in an individual’s lifetime), evolution (the history of this behaviour across different generations and taxa), and private experience (the subjective and personal experience of the behaviour).

1.6.2.1 Control

The causal factors behind frolicking are poorly understood, and limited by the very few studies that focus on this behaviour. Guhl (1958) anecdotally reported that a disturbance, such as turning the lights on or filling the feed troughs, led to an increase in frolicking and sparring. Similarly, Dawson (1962) agree that chicks disturbed by a loud noise also increased their frolicking. This would fit within a prediction of play outlined by Špinka et al. (2001; Prediction 16), that play “increases in frequency after animals move between habitats, experience substantial changes in habitat that affect locomotion, or encounter mildly frightening or novel stimuli”. A number of species show an increase in play following some disturbance to their environment (reviewed in Špinka et al., 2001). Novel objects may also stimulate certain play-like behaviours. In an unpublished trial, Keeling and Zimmerman (2009) housed small groups of broilers in either enriched (woodshavings + scattered wheat + perches), normal (woodshavings) or barren (no woodshavings or additional enrichment) pens. They found that more play behaviour (play-fighting, play-running, and play-running+wing-flapping) was observed in the barren condition compared to the enriched condition when broilers were given novel objects. The authors relate this to the increase in novel object interaction seen in pigs housed in barren conditions (Bolhuis et al., 2005), however the lack of play in the treatment with perches may have been due to space limitations, as discussed with sparring. The broilers were then provided with toys (small toothpicks, a ball, a cardboard box) for 30 minutes for each observation. Again, they found less play-running (frolicking) when birds had toys in the enriched condition compared to the barren condition. However, broilers did exhibit more play when given the toys, which suggests that play can be stimulated with novelty.

1.6.2.2 Development

The most detailed descriptions of frolicking development are found in Guhl (1958) and Dawson and Siegel (1967). Guhl (1958) reported that social behaviour in laying hen strains of domestic chick develop in the following order: escape (fear) reactions, frolicking, sparring, aggressive pecking, avoidance and fighting. He found that escape reactions were common from 3 days of age and could be stimulated easily by moving anything above the chicks. There was variation in the speed with which chicks immediately responded to the fear stimuli, but all chicks in the group quickly began to run around the pen. During week 1, frolicking behaviour was observed and it was exhibited contagiously in a flock. During week 2, frolicking bouts would lead to sparring bouts, in which chicks would mimic adult fights but without delivering any blows. Avoidance behaviours and aggressive fighting appeared to develop in week 5 and 6 (Guhl, 1958). Dawson and Siegel (1967) were in agreement that frolicking appeared prior to sparring in laying chicks, however their timings differed. They found that frolicking appeared in the first week and then increased until week 3 when it began to decline. Sparring began to appear later than frolicking and surpassed frolicking by about 25 days of age. It peaked during week 4 and then began to decline (Dawson and Siegel; 1967; extended information in Dawson, 1962). This would incidentally follow the pattern of play development in Špinka et al. (2001; Prediction 21), in which a peak in locomotor play precedes a peak in social play. The considerable difference between older and modern poultry strains, in terms of their genetics and behaviour, mean that these references are likely to be outdated. A slightly more recent paper found that frolicking was more frequently displayed in a laying hen strain compared to broilers (Mench, 1988), which is likely to be due to their overall lower levels of activity.

1.6.2.3 Adaptive function

Frolicking is a spontaneous behaviour and appears to serve no immediate function. The flapping and frenetic movements involved mean it would be an inefficient and ineffective method of fleeing a predator. Dawson (1962) agrees with Guhl (1958) in that frolicking can be stimulated by a sudden stimulus, for example turning on lights or a loud noise, but adds that there is an initial suppression of activity followed by contagious frolicking. This suggests that frolicking only occurs once it becomes apparent that there is no immediate danger, and is not a functioning escape reaction. Within Špinka et al.'s (2001) framework of play, the main function of locomotor play is to rehearse patterns of behaviour that could be disrupted by external factors and to train the animal to regain their faculties quickly. For example, when fleeing from a predator the animal will try to use the most efficient pattern of escape, however rapid changes to the environment, visual input, conspecific reactions and predator behaviours can cause unpredictable interruption and disorientation. By practising atypical movements through play behaviour, the animal is more likely to recover quickly and avoid attack. In addition to developing motor skills, Špinka et al. (2001) propose that this type of play allows animals to cope better emotionally with sudden shocks, including being faced with an unexpected predator.

Domestic fowl can still show strong fear responses in commercial conditions (Jones, 1996) and in agreement with Špinka et al.'s (2001) theory, it is possible that frolicking developed as a means to rehearse and develop escape skills. In this case, it may be interesting to explore the link between frolicking and fear responses or physical skills, for example length of tonic immobility or "righting" abilities. This link has already been investigated with food-running in laying hens (Dossey, 2009). It was predicted that hens stimulated to perform food-running would show improved body condition and lower fear responses. Hens that had received "worms" to play with were significantly heavier than those without worm experience at 10 weeks old. In a combination of fear tests, including handling, tonic immobility, novel object and open field tests, the effects of providing a "worm" were mixed. As frolicking is considered by Guhl (1958) to be an incipient agonistic encounter that precedes sparring, it is also possible that frolicking developed as a way to develop leaping and avoidance skills needed for adult fighting. As a result, frolicking in young birds could also be related to increased skills in these behaviours in adult birds.

1.6.2.4 Evolution

Frolicking has been vaguely described in other Galliformes, including partridges (Goodwin, 1953), junglefowl (Kruijt, 1964), turkeys (Sherwin and Kelland, 1998), ducks (Lee et al., 1992), geese (Lorenz; in Nice, 1943) and game birds (Wiley, 1963). Goodwin (1953) observed red-legged partridges performing escape reactions without any stimulus; running at full speed, making short flights and sudden turns (cited by Ficken, 1977). Geese and ducks were also observed spontaneously performing behaviours normally used to escape from a bird of prey when in the open (Lorenz, in Nice, 1943; cited by Ficken, 1977). More recently, in a study on time budgets in turkeys, an abnormal “running” behaviour is described where turkeys will spontaneously run in circles with their wings raised (Sherwin and Kelland, 1998). This behaviour is likened to frolicking and cautiously suggested to be a play. Frolicking in turkeys reduced between 4 – 12 weeks of age and was rarely seen after 12 weeks. In female ducks caged at 14 weeks old and observed until 55 weeks, frolicking was considered to be a comfort behaviour (Lee et al., 1992). Occurrence of frolicking in these ducks actually increased over the course of the study, possibly because the ducks became more accustomed to the cage. It is likely that frolicking is labelled differently in other studies, for example a similar behaviour is referred to as “movement flapping” by Black and Hughes (1974) and “play-running+wing-flapping” in a recent Welfare Quality Report for broilers (Keeling and Zimmerman, 2009). As such, there is a lack of detailed comparative research between species, however it is possible that frolicking is a shared trait within this order.

1.6.2.5 Private experience

As with all animals, evidence on the way poultry experience their conditions is elusive, especially concerning positive emotions. Gakel calls have been used in poultry to demonstrate frustration (Zimmerman and Koene, 1998), however no specific vocalisation has been identified in anticipation of a positive experience (Zimmerman et al., 2011). Significantly more comfort behaviours, including preening, wing-flapping, feather ruffling, body scratching and yawning were observed in birds expecting a positive reinforcement of mealworms rather than a negative reinforcement of water spray (Zimmerman et al., 2011). The guidelines for assessing welfare in broilers outlined in the recent Welfare Quality protocols include the use of qualitative behavioural assessment (QBA). QBA is an intuitive measure of an animal’s state using qualitative descriptors (e.g. calm, positively occupied, comfortable) that are scored on a scale depending on how the human observer perceives their behavioural expression (Welfare Quality, 2009). This method of assessing behaviour may be useful in future examinations of frolicking behaviour and its possible positive associations.

1.6.3 Food-running

Food-running, or “worm-running” is a conspicuous behaviour seen in chicks as early as 2 days old. A chick will pick up an object and run with it, making loud and repeated peeping noises. This object is typically rod-shaped or ‘worm shaped’ and can be nutritive or non-nutritive. Other chicks usually chase the bird and the object can be snatched and move through the group, with different birds in possession of the item then performing food-running themselves. Some chicks are more successful at holding onto the “worm” than others. A bout of food-running usually ends when the object is lost, eaten, or the birds lose interest and engage in other behaviours (Kruijt, 1964; Rogers and Astiningsih, 1991; Cloutier et al., 2004; Dossey, 2009). Earlier observations of food-running focused on very young chicks, however food-running can be stimulated equally in week 2 and week 10 (Cloutier et al., 2004).

Although observations of food-running have been sparse, several explanations for this behaviour have been debated. Kruiijt (1964) argued that the obvious functional explanation that food-running is related to food competition is incomplete, because it occurs in birds raised in isolation (Spalding, 1873; Brückner, 1933) and before any pursuing response develops. Indeed, the behaviour is extremely conspicuous and birds quickly learn to run towards the focal bird, making it unlikely that food-running is a way to prevent conspecifics from stealing food. Instead, Kruijt (1964) proposed that the primary function in young chicks may be to attract other birds to immobilise any prey too large for immediate consumption. However, food-running can easily be stimulated by any rod-shaped material, such as pipe cleaners, and chicks in possession of the ‘worm’ will immediately run to avoid conspecifics (Rogers and Astiningsih, 1991; Cloutier et al., 2004; Dossey et al., 2009). In addition, even individually tested chicks given a mealworm will perform food-running, and hunger does not appear to be a main motivating factor; birds with access to ad libitum food will still perform food-running and the mealworm that elicited the behaviour is not always eaten (Rogers and Astiningsih, 1991; Cloutier et al., 2004). Preventing conspecifics from stealing food in older chicks capable of immobilising prey themselves was suggested as a secondary function (Kruijit, 1964). If this behaviour in young chicks is related to their future ability to preserve food in relation to the unpredictable actions of other flock mates, then this behaviour could also be considered with Špinka et al.'s (2001) play framework. Food-running has been used to test social rank with varying success (Rogers and Astiningsih, 1991; Cloutier et al., 2004), however Cloutier et al. (2004) concluded that food-running more closely resembles play than serious competitive behaviour, and suggested its possible use as a welfare indicator. Indeed, food-running closely resembles forms of social object play reported in other bird species, usually taking the form of "tug-of-war" games (reviewed in Diamond and Bond; 2003). Diamond and Bond (2003) describe how "the best evidence of social object play is provided by contests over items that cannot be otherwise turned to useful purposes. Role reversals are common in social object play, and the interaction often ends with the contested item simply being discarded"

1.6.4 Play conclusions

By treating play research as a side-step into anthropomorphism or an explanation only applicable as a last resort, it is likely that our knowledge of positive behaviours in poultry has suffered. For example, many studies may have classified frolicking as "running", or excluded the strange behaviour all together. Although information on sparring, frolicking and food-running is sparse, we can compare our knowledge of these behaviours with current well-accepted definitions of play. Burghardt (2005) set out five criteria that, if met, should indicate the presence of play in all species: these criteria state that play is (1) incompletely functional in the context in which it appears; (2) spontaneous, pleasurable, rewarding, or voluntary; (3) differing from other more serious behaviours in form (e.g., exaggerated) or timing (e.g., occurring early in life before the more serious version is needed); (4) is repeated, but not in abnormal and unvarying stereotypic way (e.g., rocking); and (5) is initiated in the absence of severe stress. Current evidence available suggests sparring, frolicking and food-running can satisfy several of these criteria.

The function of sparring could be debated (for example, if threatening was considered a method of avoiding aggression), however the absence of injurious contact and clear submissive avoidance suggests it lacks an immediate function. Sparring appears to occur spontaneously, and often at the end of a frolicking bout. Sparring differs from adult aggression in that pecks tend to be brief and gentle, and any contact does not cause injury or avoidance behaviours in the recipient. Sparring is apparent throughout broilers short lives and is repeated but contains no stereotypical motions. For the fifth criteria, additional research is needed, however there is some indirect evidence that a reduction in sparring occurs in suboptimal conditions (e.g. feed restriction and a lack of space).

Frolicking appears to lack function and is a spontaneous and contagious behaviour. It differs from true escape reactions in its exaggerated flapping and chaotic movements, and occurs in the absence of any true or apparently perceived threats. Frolicking is a repeated but not stereotypical behaviour, although more research is needed to confirm its frequency in individual birds. As with sparring, whether frolicking satisfies the fifth criteria is difficult to verify and further work may shed light on whether frolicking is reduced in stressful situations.

Current functional explanations given for food-running are not readily supported by the (sparse) evidence. The behaviour is voluntary and appears to be either self-rewarding or an incomplete form of an adult behaviour used to avoid conspecifics during food-competition. The behaviour is repeated and appears to be easy to stimulate in older chicks up to 10 weeks of age. Further research is needed on the ability of this behaviour to act as a welfare indicator.

1.7 Environmental Enrichment

Environmental enrichment refers to a physical, sensory or social change made to a captive animal’s environment with the goal of improving health, behavioural repertoire and/or mental well-being (King, 2003). The concept of environmental enrichment is broad, ranging from social contact with conspecifics (Hubrecht, 1993) to provision of rubber toys (Belz et al., 2003). Providing animals with a more complex environment results in, among other things, better problem solving (de Jong et al., 2000), a reduction in stereotypical behaviours (Nørgaard-Nielsen et al., 1993), improved cognition (Bredy et al., 2003), and increased activity levels (Beattie et al., 1995). For farm animals, additional goals of environmental enrichment are to improve the public image of farming, discriminate “higher welfare” products and avoid any decline in production parameters (Newberry, 1995). Application of the term “environmental enrichment” to situations where there appears to be no obvious benefit to the animal has been criticised (Newberry, 1995), however rather than create a new phrase, this expression has been adopted as a practical way to describe an increase in environmental complexity (Jones, 1996). For this thesis, environmental enrichment refers to the latter basic definition.

1.7.1 Environmental enrichment for broilers

Newberry (1995) states that environmental enrichment should “improve the biological functioning of animals”, and as such, the ultimate aims of environmental enrichment differ depending on the species. For broilers, improvements in activity levels, group distribution, leg health, fear responses, and behavioural repertoire tend to be favourable outcomes of environmental enrichment research. Where enrichments have not yet been tested on meat chickens, results from studies of laying hens and other domestic fowl are tentatively extrapolated to broilers, until disproved. EU legislation obliges broilers to be provided with litter and outlines requirements for their photoperiod, however there is no EU or UK legislation requiring additional environmental enrichment. Producers rearing broilers under welfare assurance schemes or for particular retailers comply with additional standards; in the UK these tend to require inclusion of some combination of natural light, reduced stocking densities, straw bales and perches. For example, broilers raised on farms that follow the RSCPA Assured scheme (RSPCA, 2017b) must be housed with natural light and at no more than 30 kg/m^2. For each 1 000 birds under this scheme, there must be at least 2 m of perch space, 1.5 long-cut straw bales at all times and 1 hanging object (e.g. wooden blocks, cabbages). Birds supplied for M&S Oakham™ range must be provided with natural light and reared at 30 kg/m^2, if thinning is permitted (partial depopulation before full slaughter age) and 34 kg/m^2 if there is no thinning (M&S, 2015). Birds must also be provided with straw bales, although the type and quantity are not specified. In Northern Ireland, enriched housing of this type typically includes 2 short-cut plastic wrapped bales per 1 000 birds per cycle.

1.7.1.1 Straw bales

Despite straw bales becoming a fairly typical installation in “higher welfare” broiler housing, there is a surprising lack of scientific study on their attractiveness, effectiveness and the optimal levels of provision. The inclusion of 1.5 long-cut straw bales per 1 000 birds in the RSPCA Assured scheme (then Freedom Food) in the 1990s was based on suggestions and educated guesswork (RSPCA, personal communication, 2016). A succeeding study found positive behavioural effects of providing long-cut straw bales in a commercial broiler house (Kells et al., 2001). The straw bales were included at a density of 1 per 17 m^2, which equated to 118 bales in one house and 81 in another. The authors found that when provided with straw bales, broilers spent more time standing and walking, and less time sitting and resting in areas away from the bales. However, this density of enrichment differs from the (unchanged) RSPCA protocol and from current commercial practices. As stated, the RSCPA Assured scheme requires 1.5 bales per 1 000 birds, which is equivalent to around 33 bales per house, or 1 bale per 41 m^2 in an average house of 22 000 broilers (RSPCA, 2017b). A recent study in Germany looked at both conventional systems and enriched housing that incorporated 1.7 long-cut straw bales per 1 000 birds, which was 54 straw bales in total per house and 1 bale per 37 m^2, in addition to perches and pecking stones. Although no direct comparisons were made, numerically less resting and lying was observed in the enriched housing than the conventional housing (Bergmann et al., 2017). In a smaller scale Japanese study, broilers housed with hay bales and perches showed more standing and locomotion behaviours than those in barren conditions (Ohara et al., 2015).

An alternative to long-cut straw bales are short-cut plastic wrapped straw bales. These bales are the type typically used as enrichment bales in Northern Ireland and are considered to have biosecurity advantages. Both plastic sides of the bale are cut open to give birds access to the straw, which the birds scratch and peck at. Unlike long-cut straw bales, these short-cut bales are fully dismantled by the birds, suggesting a more interactive but time-limited enrichment. Short-cut bales are limited in their additional value as a perching enrichment because the bales degrade rapidly and are more unstable. However, a main advantage of short-cut bales is that the birds are able to actively forage and spread out the straw, which improves foraging opportunities and enables the “self-spreading” of dry bedding. Provision of these bales varies between farms, however, as these bales are destroyed by the broilers over time, they tend to be cut open in a staggered manner which means less usable bales are available at any one time. The use of these bales has only recently been scientifically investigated. When 30 short-cut bales were included in a commercial house, which is approximately one per 44 m^2, and cut open in a staggered manner throughout the experiment, there were no effects on broiler resting behaviour or levels of activity (Bailie et al., 2013). However there was an improvement in latency to lie when broilers had straw bales, which suggests a positive effect on leg health. A further investigation on the possible effect of density of bales found no difference in activity levels between birds given 30 or 45 bales per house, which equates to 1 per 44 m^2 and 1 per 29 m^2 respectively (Bailie and O’Connell, 2014). In addition, better latency to lie scores were recorded when birds had 30 bales rather than 45. It may be that the difference between bale densities was too small to create a change in overall time budgets. This trial also differs from other bale research in that natural light was provided throughout, which is likely to have had more of a significant effect on behaviour (Bailie et al., 2013). However, the lack of difference in any production parameters between the two treatments also suggests no negative impact of increasing bale density on commercial output. More research is needed on how bale density and type are able to influence broiler activity levels and leg health.

Straw bales also appear to have significant protective value to broilers in commercial housing. Authors consistently report that birds cluster around the base of straw bales (Kells et al., 2001; Bailie et al., 2013; Bergmann et al., 2017). When bales are present, this behaviour is observed early in the rearing period, with chicks grouping around bales and sleeping huddled together (Bergmann et al., 2017). Seeking cover is consistent with the natural behaviour of fowl, who prefer to perform behaviours that make them more vulnerable to predation, such as resting, in the presence of shelter (Wood-Gush et al., 1978)

1.7.1.2 Perches and barriers

Perching is an innate predator avoidance behaviour in junglefowl (Collias and Collias, 1967). In natural conditions, chicks are brooded on the ground and then follow the mother onto perches by 6 weeks of age (McBride et al., 1969). This behaviour has persisted in domesticated fowl, with chicks raised without a mother beginning to use perches within the first few weeks (LeVan et al., 2000; Heikkilä et al., 2006). Studies of laying hen behaviour found that birds will consistently choose the highest perch (Olsson and Keeling, 2000) and display signs of frustration if prevented from roosting (Olsson and Keeling, 2000; Olsson and Keeling, 2002b). For broilers, low levels of perch use are frequently reported (LeVan et al., 2000; Rodriguez-Aurrekoetxea et al., 2015; Norring et al., 2016; Bergmann et al., 2017). However, significantly more broilers will use a raised platform compared to a simple bar perch, which suggests that it is their ability to balance on a bar, rather than motivation to perch, that is a limiting factor in broiler roosting (Norring et al., 2016, Bailie et al., 2018; Kaukonen et al., 2017a). Broilers body weight and anterior centre of gravity are likely to make it difficult to climb onto bar perches and remain balanced. Broilers are also more likely to use flat perches rather than those at a 10^{o} or 20^{o} angle, which may be because of the extra stability that a flat perch provides and the additional effort required to reach the top of an angled perch (LeVan et al., 2000).

It has been suggested that, in addition to satisfying a natural behaviour, jumping on and off perches may be a form of exercise for broilers. The use of barriers and ramps in front of feeders and drinkers may also necessitate more walking and effort from broilers to reach feeders and drinkers. An increase in exercise in broilers has been linked with improved leg condition (Reiter and Bessei, 1995), and as such it has been proposed that perches and barriers could improve broiler leg health. However, the literature is fairly inconsistent. Broilers that had to negotiate barriers to reach feeders and drinkers had wider and improved asymmetry of the tibia, suggesting an improvement in bone strength and stability (Bizeray et al., 2002a; Ventura et al., 2010). Provision of barriers has also resulted in more activity and less lying behaviour (Bizeray et al., 2002b). A reduction in footpad dermatitis has been found in broilers housed with perches, presumably because they spend less time in contact with the damp litter (Ventura et al., 2010; Kiyma et al., 2016). However, there have also been reports of perches and barriers having no effect on tibia length (Ventura et al., 2010), footpad dermatitis (Bench et al., 2016), fluctuating asymmetry (Bizeray et al., 2002a), activity levels (Rodriguez-Aurrekoetxea et al., 2015) and walking ability (Su et al., 2000; Bailie and O'Connell, 2015). Indeed, ramps and barriers have been employed to experimentally induce lameness and bacterial chondronecrosis in broilers by creating excessive mechanical stress on their joints (Gilley et al., 2014; Wideman et al., 2015). Inclusion of perches that are infrequently used may also increase stress by reducing the available floor space (Heckert et al., 2002). New research is currently pointing to the value of platform perches over traditional perches for broilers. Broilers with access to platforms, compared to traditional perches, showed improved gait scores and tibial dyschondroplasia measures (Kaukonen et al., 2017a).

1.7.1.3 Artificial cover

In line with their wild ancestors, broilers show a reluctance to enter large open spaces and when free ranging will demonstrate a preference for areas with vertical tree and shrub cover (Dawkins et al., 2003). There is very little vertical cover available in a commercial house and broilers show a tendency to group near pen walls (Newberry and Hall, 1990). When artificial cover was provided to laying hens in the form of vertical plexiglass panels, more birds were observed in covered areas and there was an increase in resting and preening in the presence of cover panels (Newberry and Shackleton, 1997). This is consistent with birds seeking out cover to perform vulnerable activities that may obscure their vision. In addition, birds were more attracted to areas with partially opaque cover panels instead of opaque panels, appearing to prefer partial concealment which could still allow for identification of a nearby predator (Newberry and Shackleton, 1997).

In broiler housing, providing similar mesh panels resulted in a more even distribution of birds (Cornetto and Estevez, 2001a) and an increase in resting in central areas (Cornetto and Estevez, 2001b). There was a reduction in foraging in pens with vertical panels and an increase in dustbathing as the group size increased (Cornetto and Estevez, 2001b). Birds grouping near vertical cover and pen walls were also less likely to be disturbed while resting compared to those in open areas (Cornetto et al., 2002). However, a recent study conducted at a commercial level on free range broilers found no effect of indoor panels on resting or comfort behaviours, with only a slight increase in locomotion in central areas of houses with panels (Rodriguez-Aurrekoetxea et al., 2015). It may that the extra space available in free range housing or the low density of enrichments limited their impact, however more research is needed at a commercial scale to clarify the benefits of artificial cover.

1.7.1.4 Pecking enrichments

Although no legislation sets criteria for pecking enrichments, other than bedding, the RSPCA Assured scheme requires broilers to have one pecking object (for example a Peck-a-Block, brassica or wooden block) per 1 000 birds (RSCPA, 2017b). Pecking enrichments have been studied extensively in laying hens because of the link between thwarted pecking behaviours and injurious feather pecking (Huber-Eicher and Wechsler, 1998; Johnsen et al., 1998; Dixon et al., 2008). Laying hens show a preference for bunches of string over chains, beads, baubles, feathers and string with beads (Jones et al., 1997; Jones et al., 2000) Presenting these enrichments together also elicits more pecking behaviour than when they are presented singly (Jones et al., 2000). White and yellow string are more attractive than blue and orange string, and string continues to be attractive after repeated exposure (Jones and Carmichael, 1998). Broilers showed little interest in bunches of string when presented in bedded pens alongside dustbathing trays (Arnould et al., 2004), however the birds may not have been motivated to find alternative pecking stimuli in the presence of woodshavings and sand. Broiler breeders show an initial interest in bunches of string but quickly become habituated to their presence (Hocking and Jones, 2006). Recently, a greater attraction to string has been found in commercially housed broilers, with one bout of pecking occurring every 78 seconds at each piece of white string (Bailie and O'Connell, 2015). In addition, broilers housed with a cereal based pecking enrichment called a Pecka-Block™ show improved feather condition, less ground pecking and increased dustbathing (Guy and Wright, 2003). Broilers interest in a pecking enrichment may be limited by their general reduction in pecking and foraging behaviours compared to layer hens, however additional research is needed in commercial houses to confirm their effectiveness.

1.7.1.5 Dust baths

Dust baths have not been introduced into commercial broiler systems in the UK and there appear to be no commercial scale studies looking at the use of dust baths in broiler housing. As mentioned, sand and peat are preferred substrates for dustbathing (Petherick and Duncan, 1989; Shields et al., 2004). These materials are unlikely to be appropriate for inclusion in UK broiler housing because peat is considered to be environmentally unsustainable and expensive, and sand interferes with the process of litter disposal. However, both of these materials have historically been used in the UK, and continue to be used as commercial broiler bedding in other countries. For example, peat is the most commonly used broiler bedding in Finland and appears to improve the incidences of footpad dermatitis and hock lesions (Kaukonen et al., 2017b). Sand bedding is also used as an alternative to woodshavings in some areas of America (Grimes et al., 2002) and has been linked with increased body weight and improved dermatitis (Bilgili et al., 1999; Bilgili et al., 2009). However there is little information available on the behavioural effects of raising broilers entirely on sand or peat at a commercial scale. A more even distribution of birds and increase in foraging was achieved by adding sand trays to pens of broilers (Arnould et al., 2004). Including a sand section in a broiler pen also resulted in improved meat quality and reduced contact dermatitis (Simsek et al., 2009). However, when broilers were housed in pens of sand compared to woodshavings there was no difference in the frequency of any behaviours, which could suggest that broilers have a fairly inflexible time budget (Shields et al., 2004).

1.7.1.6 Light

Poultry have a highly specialised visual system and several components of commercial lighting have been shown to influence broiler health and behaviour. The continuous or near continuous lighting programmes that were traditionally used to rear broilers have been associated with several welfare issues, including the development of eye abnormalities (Oishi and Murakami, 1985), higher mortality levels (Classen et al., 1991) and increased tibial dyschondroplasia (Sorensen et al., 1999). Daylength also influences broiler behaviour, with the amount of standing, walking, foraging and dustbathing reducing as daylength increases (Schwean-

Lardner et al., 2012a). In addition to several welfare benefits, providing broilers with an extended period of darkness also improves productivity (Schwean-Lardner et al., 2012b). As a response to these issues, EU regulations now require broilers to be raised under a 24 hour lighting rhythm from 7 days of age until 3 days before slaughter, with a minimum of 4 hours of uninterrupted darkness and a total of 6 hours of darkness every 24 hours (Council Directive 2007/43/EC).

Light intensity is also regulated, with a minimum light intensity of 20-lux required over 80% of the usable space (Council Directive 2007/43/EC). Young broilers show a preference for brighter areas, while older broilers will choose dim areas, which is likely to be due to the large amount of time older broilers spend resting (Davis et al., 1999). When raised in varying light intensities, there is no difference in body weight or immune response, however broilers are less active in lower light intensities and showed a less pronounced difference between day and night activities (Blatchford et al., 2009). Intensive broiler houses are typically lit by either incandescent bulbs or fluorescent strip lighting, however windowed houses that provide natural light are becoming more common in the UK. Natural light is a requirement for birds reared under the RSPCA Assured scheme (e.g. RSPCA, 2017b) and for particular retailers (Oakham™ range; M&S, 2015). There have been few studies on the effects of rearing broilers inside with access to natural light. Broilers housed in pens with natural light were found to be less active and perform less dustbathing, play and foraging behaviours (Ruis et al., 2004). However, in commercial housing natural light reduced time spent resting, and improved gait scores, latency to lie and litter condition (Bailie et al., 2013).

1.7.1.7 Stocking density

Broiler stocking density refers to the number of birds housed in a unit of floor area, usually expressed as the total weight of birds per m^2. In the EU, broilers can be stocked at a maximum of 33 kg/m^2 unless additional criteria are met (Council Directive 2007/43/EC). These include limits on ammonia, temperature and humidity levels, in addition to enhanced communication and record keeping. An application to increase the stocking density further to 42 kg/m^2 can then be made if these criteria are met, there has been no failure to comply with any EU regulations, and there have been consistently low mortality rates. Welfare assurance schemes also usually limit stocking density, for example there is a 30 kg/m^2 limit for the RSPCA Assured scheme and a 38 kg/m^2 limit for the Red Tractor Assurance scheme (Red Tractor Assurance, 2017; RSPCA, 2017b). Own brand retailers may have additional requirements, for example M&S do not allow stocking density of above 34 kg/m^2 (Oakham™ range; M&S, 2015).

High stocking densities have been associated with a number of broiler welfare issues. When stocked at 40 kg/m^2 compared to 34 kg/m^2, broilers had higher mortality, an increase in dermatitis and leg problems, and more disturbed resting periods (Hall, 2001). Lower body weights (Proudfoot et al., 1979; Dozier et al., 2006) and a reduction in locomotion, distance moved and foraging behaviours are also seen in higher stocking densities (Blockhuis and Van der Haar, 1990; Lewis and Hurnik, 1990). The use of platform perches and woodshavings bales declined when broilers were stocked at 30 kg/m^2 compared to 25 kg/m^2 (de Jong and Goërtz, 2017), suggesting enrichment use could be affected even at relatively low stocking densities. Estevez (2007) suggests that broiler productivity and welfare can be maintained if broilers are stocked somewhere between 34 kg/m^2 and 38 kg/m^2, however Dawkins et al. (2004) argue that other factors have significantly more impact on broiler welfare. Although very high stocking densities appear to be unequivocally detrimental, Dawkins et al. (2004) found that good stockmanship, especially in maintaining litter and low ammonia levels, was a more important indicator of many welfare indices.

1.8 Rationale for research

In response to the growing demand for high welfare animal products and the poor image of intensive farming (Mayfield et al., 2007; EU Commission, 2015; Cornish et al., 2016), broiler producers have increased the complexity of some of their intensive housing systems (e.g. RSPCA, 2017b; M&S, 2015). There is limited commercial scale research available to support these modifications, however introduction of straw bales and perches have been found to improve broiler activity levels and leg disorders (Kells et al., 2001; Bizeray et al., 2002a; Ventura et al., 2010). There is a financial incentive for producers to make further improvements to intensive broiler housing, and scientific investigations into broiler enrichment will be needed to provide evidence based recommendations for future housing designs.

Although dustbathing has been identified as an important behaviour for domestic fowl (Vestergaard et al., 1997; Zimmerman et al., 2000; McGrath et al., 2016), little is known about the levels of dustbathing performed by commercially housed broilers. Providing a dustbathing material has the potential to improve welfare by satisfying a natural motivation and giving birds an opportunity to exercise. Increasing the complexity of intensive housing has also been associated with positive emotion in farm animals (Douglas et al., 2012; Carreras et al., 2016). Studying enriched broiler housing therefore gives us an opportunity to investigate positive welfare indicators in poultry. Improvements to intensive broiler housing are likely to have a significant impact on animal welfare, with 62 billion broilers produced in similar systems worldwide (FAOstat, 2014). In addition, understanding the effect of farm environments on animal emotion would have important implications for both science and society.

1.8.1 Research aims

The overarching aim of this thesis is to determine whether provision of a dustbathing material would improve the welfare of intensively farmed broiler chickens. To that end, the individual aims are to:

1) Determine the extent to which modern broilers would use a dustbathing material in commercial housing, and identify an attractive dustbathing substrate that would be suitable for commercial conditions.

2) Explore the benefits of including dust baths as an alternative or supplementary enrichment for commercial broiler chickens, and further determine whether broilers would be more attracted to enrichments if they were grouped into "enrichment areas" rather than provided individually.

3) Compare the levels of play behaviour and fearfulness in broilers housed with or without perches and dust baths, in order to better understand the effect of these enrichments on broiler mental well-being.

All methods described in this thesis were approved by the School of Biological Sciences (Queen's University Belfast) Research Ethics Committee (reference number QUB-BE-AREC-17-001).

Chapter Two

Study 1

An evaluation of potential dustbathing substrates for commercial broiler chickens

Abstract

Provision of an appropriate dustbathing substrate may allow broiler chickens to satisfy a natural motivation and give them an opportunity to exercise. The main aim of this study was to evaluate the extent to which different substrates promote dustbathing behaviour in broilers. The trial was replicated over three production cycles in one commercial broiler house, with approximately 22 000 Ross broilers (Aviagen Ltd, UK) housed per cycle. The birds were provided with access to five experimental substrates from day 10 of the 6 week production cycle. The substrates included the following: 1) peat (P), 2) oat hulls (OH), 3) straw pellets (SP), 4) clean woodshavings (WS), and 5) litter control (C). The substrates were provided in fifteen steel rings (1.1m in diameter, three rings per substrate) dispersed throughout the house. The level of occupancy of the rings, behaviours performed in each substrate, and the effect of ring position (central or edge of house) were assessed in weeks 3, 4, 5 and 6 using scan sampling from video footage. Where substrates successfully promoted dustbathing, the length and components of the bouts (including number of vertical wingshakes and ground pecks) were also assessed. Results showed that birds used P significantly more than the remaining substrates for dustbathing (P < 0.001). Oat hulls were the second most preferred substrate for dustbathing, with significantly more birds dustbathing in the OH compared to SP, WS and C (P < 0.001). The least sitting inactive was also seen in the P and OH rings compared to the SP, WS and C (P < 0.001). The highest levels of foraging were recorded in the P, OH and WS compared to SP and the C. Position of the rings did not affect the types of behaviours performed in any substrate, although overall more birds were counted in the central compared to edge rings (P = 0.001). More detailed information on dustbathing behaviour was only recorded in the P and OH treatments, and there were no differences in the length of dustbathing bout, or components of the bout between them (P > 0.05). The use of OH is likely to be more environmentally sustainable than that of P, and our results suggest that this substrate is relatively successful in promoting dustbathing. However a preference was still observed for P and further work should investigate whether other suitable substrates could better reflect its qualities.

2.1 Introduction

Dustbathing is a distinctive behaviour observed in many bird species and has been well documented in both Red Jungle Fowl and modern chickens (Kruijt, 1964; van Liere et al., 1991). With access to litter, birds will perform dustbathing approximately every second day (Vestergaard, 1982), with the individual elements of the behaviour developing in younger birds until the sequence becomes fixed around 10-12 days old (Kruijt, 1964). A dustbathing bout usually begins with a bird scratching at the ground and raking dust closer to their body, before squatting with their feathers erect. The bird then kicks dust into their feathers by scratching their legs and performing vertical wing shakes, before rubbing their head along the ground and stretching their legs. A dustbathing bout usually ends with the bird standing and shaking excess substrate off their body (van Liere et al., 1991).

Thought to function to maintain feather condition and remove ectoparasites (van Liere and Bokma, 1987; Martin and Mullens, 2012), dustbathing has proved to be highly motivated and birds demonstrate observable frustration when prevented from performing the behaviour (Vestergaard et al., 1997). Despite this, the level of dustbathing reported in commercial broilers is usually very low, matching a generally low level of foraging and locomotion in these birds (e.g. Bailie et al., 2013). This may reflect a reduced physical capacity, and probably motivation, to perform active behaviours without stimulation in birds genetically selected for high productivity (Lindqvist, 2008). Low levels of dustbathing may also reflect a lack of a suitable substrate in the house. While bedding is provided in commercial systems, the typical consistency of the litter and the fact that it tends to become wetter and more compact across the production cycle, may limit its attractiveness for dustbathing. Broiler chickens may therefore be experiencing frustration from a lack of suitable substrate, and providing birds with a preferred dustbathing material that is compatible with commercial systems may be an effective environmental enrichment.

Domestic fowl display preferences for dustbathing materials and consistently choose loose, friable substrates, which may reflect their effectiveness at removing lipids. Although previous experience may influence a bird's perception to an extent, identifying suitable dustbathing substrates appears to be innate and adult birds will still show a preference for substrates they have no previous experience of (Sanotra et al., 1995; Wichman and Keeling, 2008). Peat has been identified as a highly preferred substrate to laying hens (Petherick and Duncan, 1989; de Jong et al., 2005; de Jong et al., 2007) and is thus a frequently used stimulant in trials investigating dustbathing (e.g. Wichman and Keeling, 2008). Sand also appears to be beneficial and highly attractive to broilers (Shields et al., 2004; 2005). Other substrates that have been tested in dustbathing trials with less success include rice hulls, woodshavings, shell sand and paper (Shields et al., 2004; Toghyani et al., 2010, Guinebretière et al., 2014; Villagrá et al., 2014). The quality of the dustbathing performed may also be influenced by substrate type. More vertical wing shakes and ground pecking were performed on sand compared to woodshavings (Shields et al., 2004), and dustbathing bouts were longer in peat compared to sand, sawdust and woodshavings (Petherick and Duncan, 1989).

Biosecurity restrictions prevent the use of untreated earth, and, although sand and peat are frequently used in dustbathing trials and consistently reported as optimal, sand may interfere with the processing of used litter and peat is environmentally unsustainable and expensive. This trial was designed to test the attractiveness and level of use of various substrates that would be appropriate for inclusion in commercial broiler houses. Although the primary focus was on dustbathing, other activities performed in each substrate were also recorded to determine whether they would promote additional active behaviours, such as foraging. The substrates that were evaluated included peat, ground oat hulls, straw pellets, clean woodshavings and litter (standard woodshaving bedding which degraded across the cycle and served as a control treatment). It would also be valuable to know, in a commercial house, whether level of use of a substrate varies depending on its position around the house and therefore this study also investigated the effect of location on enrichment use.

2.1.1 Pilot study

An initial pilot trial was performed in one windowed commercial broiler house in Northern Ireland over two production cycles. Approximately 23 000 mixed sex Ross 308 broilers were placed “as hatched” at the start of each cycle. On day 10, five substrates were placed in fifteen steel rings and distributed evenly around the house, in both central and outer “edge” areas of the house. All substrates were topped up to maintain their original condition throughout the trial. During weeks 2 and 3 of each production cycle, Camileo X-Sports cameras were attached to tripods and used to film each substrate for 2 hours in one randomly chosen central and edge ring. Video footage was then analysed using scan sampling, with five scans per hour of footage (at 5, 15, 25, 35 and 45 minutes). During scans, the number of birds inside each ring was counted, and bird behaviour was categorised as either dustbathing, foraging, standing, sitting, walking, stretching, sitting pecking, sitting preening, standing, preening, resting, lying, or other. These methods were applied in the main trial and are fully described in Section 2.2.

The substrates included were: 1) peat, 2) oat hulls, 3) Well-Dry, 4) straw pellets and 5) woodshavings. Substrates were selected based on previous research and advice from the producers. Peat is known to be an attractive substrate for dustbathing in laying hens, and was chosen as a useful comparison with other suggested materials. Oat hulls are a by-product of oat milling and would be a cheap and easily sourced substrate for commercial housing. Straw pellets have been used as broiler bedding and break down into a dusty material that could potentially promote dustbathing. Woodshavings were chosen to investigate whether the broilers current bedding is successful in eliciting any dustbathing behaviour. The producers had also expressed an interest in evaluating a material known as “Well-Dry”. This material is a light grey powder with a consistency similar to flour, and is typically used as a feed-additive (Sol 4 u Europe, 2014, 2016). It has additionally been suggested for use as a caking agent to reduce litter moisture and ammonia content, and as a dustbathing substrate for broilers (Sol 4 u Europe, 2014).

During the pilot trial, there was some concern that Well-Dry was increasing the levels of dust in the house, based on the dust visible in the air around the Well-Dry rings. Results from the preliminary trial indicated that Well-Dry was the least attractive substrate in terms of average birds in the rings, although the birds using the Well-Dry did appear to identify it as a dustbathing and foraging material (Table 1). The decision was made to discontinue use of Well-Dry for the main trial. It was also noted that clean woodshavings (consistently topped-up throughout the trial) did not reflect the broilers litter, especially towards the end of the trial, and did not represent a suitable control. Therefore, the substrates included in the main trial were 1) peat, 2) oat hulls, 3) straw pellets, 4) woodshavings, and 5) litter control (a ring placed on the existing litter that followed normal degradation).

Table 1. Mean birds counted in each substrate and the distribution of behaviours observed during the pilot trial

Substrate		Behaviour (%)					
	Mean birds	DB	F	Si	Lo	Pr	O
Peat	25	9	16	58	7	9	1
Oat hulls	19	12	18	53	6	10	1
Well-Dry	9	13	39	27	16	3	2
Straw pellets	24	4	5	73	8	9	1
Woodshavings	29	0	6	78	9	6	1

The behaviours recorded were: dustbathing (DB), foraging (F), sitting (Si; this included sitting inactive, sitting preening, resting and lying), locomotion (Lo; this included standing and walking), preening (Pr; this included standing preening and sitting preening), and other (O; this included stretching and other).

2.2 Material and methods

2.2.1 Subjects and housing

The main experiment was carried out between August and December 2015, in one commercial broiler house over three replicate 6 week cycles, with approximately 22 000 Ross broiler chickens (Aviagen Ltd, UK) housed per cycle. Day old chicks were placed ‘as hatched’ at the start of each cycle, and therefore there was an approximate 50:50 mix of males and females. The windowed commercial house used was a standard 19 m x 74 m metal framed shed, with a total floor area of approximately 1 398m^2, giving an initial stocking density of 16 birds/m^2. At day 30, a proportion of the birds were removed for “thinning” which is the common commercial practice of partial depopulation of the flock for slaughter, and the remaining birds were cleared between days 37 and 42.

Birds were raised under commercial management practices. Water was provided by nipple drinkers and feed was supplied ad libitum throughout rearing. Temperature and humidity were controlled automatically to maintain levels within the commercial standard. Natural light was provided through 43 windows along the long sides of the house (measuring 220 cm wide × 60 cm high, at a height of 1.5m), and artificial strip lighting was also provided. The lighting regime used followed EU regulations: time in darkness increased by 1 hour per day, from 1 hour at a day old to 6 hours on day 7, and then decreased on day 29 by 1 hour per day to 1 hour of darkness, which was maintained from day 33 to slaughter. Woodshavings were provided as bedding before the birds were placed, with additional shavings then distributed at the farmer's discretion across the cycle to maintain litter quality.

2.2.2 Treatments and experimental design

Fifteen steel rings were positioned evenly (approximately 1 per 93m^2; Figure 1) throughout the house on day 10 of the cycle. Although it appears to be preferable to include bedding materials as early as possible, for birds to properly associate them with foraging and dustbathing (Vestergaard and Baranyiova, 1996; Huber-Eicher and Wechsler, 1997), there are practical limitations under commercial conditions. Chick feeder sheets are rolled onto the ground for the first week of the cycle which prevented ring placement, and there were also some concerns that chicks could get trapped in the rings. As already outlined, chickens show an innate ability to identify 'dust' (Nicol et al., 2001) and day 10 is just within the "sensitive period" for learning in young chicks (Vestergaard and Baranyiova, 1996; Huber-Eicher and Wechsler, 1997). As this was a study designed to test the value of these enrichments in a commercial environment, it was also deemed important to present enrichments in a realistic rather than experimental manner. However, further research into any potential benefits of including a dustbathing enrichment on day 0 would be valuable.

The rings had a diameter of 1.1m and were 7.62cm deep; birds were able to climb into the rings from day 10 and were unable to perch on the ring edges. With the exception of the litter control, three rings of each substrate were cleared of litter and filled with either Irish moss-peat (P), oat hulls (OH), straw pellets (SP), or woodshavings (WS) (Photo 1). The moss-peat provided was commercially available Sphagnum peat (Better Growing Ltd, UK). Oat hulls are the ground outer hull of oats, produced as a by-product of oat milling and locally sourced (Whites Speedicook Ltd, Craigavon, UK), with a consistency and colour similar to sawdust. Straw pellets are compressed, pelleted wheat straw which can be used as an alternative bedding for broilers. The pellets degrade into a dark brown, moisture absorbent material that is also similar in consistency to sawdust. The woodshavings supplied were the same material that the birds were initially bedded on. All materials have previously been included in trials with poultry (e.g. Petherick and Duncan, 1989; Hetland and Svihus, 2001) or are used within the poultry industry. The three rings for the litter control treatment were simply placed on top of the existing woodshavings bedding and allowed to degrade into "litter" (which can involve a mixture of woodshavings, faeces and feed). The substrate locations were pre-determined to ensure the presence of each substrate in both central and edge locations of the house. Rings in edge locations were equidistant from feeders and drinkers and birds were able to reach both from the rings (Figure 1). Rings placed in central lines were further from feeders and drinkers and neither could be reached by birds inside the central rings. For each replicate, rings remained in the same location but the substrates they contained were rotated.

In order to keep the P, OH, SP and WS dry, friable and in a condition suitable for dustbathing and foraging they were replenished throughout the study. These substrates degraded at a different rate and were maintained based on their individual condition. Fresh substrate was added to the rings either when they contained $\leq$ half the original level of substrate, or when the substrate was no longer considered friable enough for dustbathing (e.g. was compacted or damp). However, regardless of condition, all P, OH, SP and WS rings were always refilled to their original level on the morning of observations to avoid novelty bias. Control rings were not refilled with woodshavings, and therefore degraded similarly to the house litter.

Moss-Peat

Oat hulls

Straw pellets

Woodshavings

Control

Well-Dry

Photo 1. Potential dustbathing substrates evaluated within this trial. Peat, oat hulls, straw pellets, woodshavings and control rings were tested in the main experiment. Well-Dry was trialled during the pilot study only.

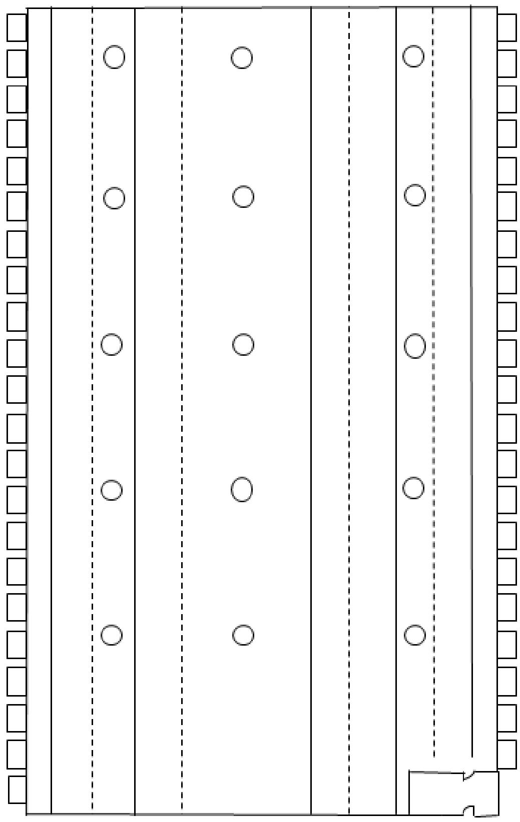

Figure 1. Representation of ring placement (circles) within the commercial broiler house. Rectangular boxes along the walls of the house represent windows. Within the house, vertical solid lines are drinker lines and broken vertical lines are feeders.

2.2.3 Data collection

The farm was visited four times per production cycle in weeks 3, 4, 5 (before thinning) and 6 (after thinning). Between 12:00 h and 16:00 h, ten rings (two of each substrate) were filmed for one hour each using five Toshiba Camileo X-Sports cameras mounted on wooden tripods. The rings filmed were chosen randomly each week, with the condition that one ring containing each substrate was located in an edge location and one in a central location. The order of filming, either edge or central ring first, was randomised each week. All data collection was performed by the same observer. Scan sampling of video recordings was used to observe birds inside the rings (Weeks et al., 2000; Shields et al., 2005). For each hour of footage, instantaneous scans were performed at 5,15,25,35 and 45 minutes. The total number of birds in the ring were counted and the behaviour of each bird was categorised according to Table 2.

Although comparison of dustbathing components was planned for all substrates, sufficient dustbathing for analysis was only recorded in peat and oat hulls rings. Comparison of the elements of dustbathing performed in peat and oat hulls was made using focal observations of 24 birds per substrate (n total = 48). These observations were performed during week 5 when the highest mean number of dustbathing bouts were performed. For each of three cycles, two videos (one central and one edge; two hours of footage) were analysed per substrate. In each video, the first four birds to perform a vertical wingshake (VWS; classic dustbathing action that shuffles the wings up and down) were identified. The video was rewound to their first VWS in each case and the rest of their dustbathing bout was analysed. The duration of the dustbathing bout was determined as the time between the first VWS and when the bird either performed a bodyshake, left the ring or performed no dustbathing behaviour for 10 minutes after the last VWS. During the bout, the number of VWS's, ground pecks, leg scratches and siderubs (rubbing the head and neck along the ground) were counted. The method that ended the bout was also recorded: either with or without a bodyshake.

Table 2. Ethogram of broiler chicken behaviours used in the present trial, based on Cornetto and Estevez (2001b) and Shields et al. (2005)

Behaviour	*Definition*
Dustbathing	Classic lying and rolling head in the substrate, accompanied with vertical wing shakes, preening, scratching and ground pecking.
Foraging	Scratching and pecking at the substrate (from a standing or walking position)
Standing	Standing with no other activity
Sitting	Sitting with no other activity
Walking	Walking, with no other pecking or scratching activity
Stretching	Stretching out a wing and/or leg and then retracting it in one motion
Sitting pecking	Sitting and ground pecking
Sitting preening	Preening, running beak through feathers, while sitting
Standing preening	Preening, running beak through feathers, while standing
Resting	Sitting with head under wing, or resting on the ground
Lying	Bird lying on one side with a leg and/or wing stretched out
Other	Any other behaviours, e.g. eating or drinking

2.2.4 Statistical analysis

For the instantaneous scan observations, counts from the five scans were pooled to give an average number of birds present in the ring (ring occupancy) and average number counted in each behavioural category, per hour. Behaviours were then grouped to facilitate analysis. “Standing” and “walking” scores were grouped into “locomotion” as both behaviours were performed from an upright position but were separate from foraging behaviour. “Sitting inactive”, “resting” and “lying” were grouped into “sitting inactive” because the motivation for these behaviours is linked and the outcome on leg health is similar. “Standing preening” and “sitting preening” were grouped in order to see the effect on overall preening behaviour. “Stretching” and “other” were excluded from analysis because they were infrequently recorded. The behaviour “other” was almost exclusively scored when birds sat inside the ring but interacted with feeders and drinkers. This was deemed irrelevant to the aims of this study and was excluded from analysis. Normality of the data was assessed through inspection of histograms, Q-Q plots and Shapiro-Wilk tests on data residuals. Where necessary, data were transformed to improve normality prior to parametric analysis, or where transformations were not appropriate non-parametric tests were applied. A significance level of $P < 0.05$ was used for all tests.

Total counts of birds using the rings were used to demonstrate the general attractiveness of substrates. This was analysed using overall counts (all weeks) and counts within weeks. The latter analysis was performed to determine if preference for substrate was affected by age. Residuals for ring occupancy counts were positively skewed and were improved with square root transformation prior to analysis with a one-way ANOVA of transformed means by "substrate type". "Cycle" was initially included within the model and was disregarded as it had no significant effect on variation between substrates. Due to one case of missing data for the oat hulls rings, a Gabriel test was chosen for post-hoc analysis to account for the unequal sample size.

To compare the behaviours performed in each substrate, analysis was carried out on both the average number of birds performing each behaviour, and the percentage of birds that they represented (in relation to the total number in that substrate ring). The average number of birds performing each behaviour showed how many birds were attracted to use the substrate, while values for the percentage use were limited to showing how much of a behaviour was performed in relation to the other birds in the ring. Results for both methods were similar and only analysis of the average number of birds is presented; percentage values are presented for interpretation. Residuals were positively skewed and improved with a square root transformation prior to analysis. For each behaviour, the overall number of birds was compared by substrate using a one-way ANOVA on transformed means. Analysis was also performed to investigate possible changes in substrate use over time. Only the percentage of birds in the ring performing different behaviours was used for analysis; this was because the average number of birds using each ring reduced over time as fewer broilers could fit in the ring. Residuals for the percentage of birds performing each behaviour by week were non-normally distributed and could not be improved by transformation. Therefore, to investigate substrate use over time, a Mann Whitney U test was used to assess whether differences were observed between weeks 3 and 6 in the percentage of birds engaged in different behaviours within each substrate type.

To investigate the effect of ring location, the average number of birds present in the rings and the percentage birds performing each behaviour were grouped by ring location; either central (n = 59) or edge (n = 60). A two-way ANOVA with "location" and "substrate" as treatment factors was used to compare location main and interaction effects on ring occupancy and proportional use. For focal dustbathing observations of peat and oat hulls, independent t-tests were used to compare bout length and components in focal observations, and the method of bout termination was analysed using a chi squared test.

2.3 Results

2.3.1 Ring occupancy

A total of 8457 broilers were observed in the rings over the course of the trial. Substrate had an effect on the mean number of birds recorded in the rings ($F_{4,114}$ = 6.740, $P < 0.001$). Overall, significantly more birds were counted in the peat and woodshavings rings compared to the oat hull and straw pellets, however there was no significant difference between the litter control and any other substrate (Table 3). Between each week, there was some variation in occupancy between substrates although the occupancy patterns tended to reflect the overall pattern of higher numbers of birds counted in the peat and woodshavings rings compared to the oat hulls and straw pellets. The higher occupancy in peat developed over time, with a clear preference for peat developing from week 5 over oat hulls and straw pellets (Table 3).

2.3.2 Behaviour in each substrate

Of all birds observed in the rings in total, 10% were observed dustbathing, 16% foraging, 18% sitting pecking, 39% sitting inactive, 6% preening and 10% were in locomotion. Substrate type had a significant effect on several behavioural categories, including the number of birds observed dustbathing ($F_{4,114}$ = 63.86, $P < 0.001$) and foraging ($F_{4,114}$ = 20.27, P < 0.001); post hoc tests are presented in Table 4. The highest levels of dustbathing were seen in peat rings. Oat hulls were the next most preferred substrate for dustbathing, with significantly more dustbathing observed in oat hulls compared to straw pellet, woodshavings and control rings. Significantly higher levels of foraging were recorded in peat, oat hulls and woodshaving rings compared to straw pellets and the control. The number of birds recorded sitting pecking ($F_{4,114}$ = 17.27, P < 0.001) and sitting inactive ($F_{4,114}$ = 15.85, P < 0.001) was also affected by substrate. The highest level of sitting pecking was recorded in the woodshavings rings, and significantly more birds were observed sitting inactive in the woodshavings, straw pellet and control rings compared to the oat hull and peat rings. Although generally low levels were observed, substrate also had an effect on levels of preening ($F_{4,114}$ = 8.84, P < 0.001), with lower levels of preening observed in oat hulls compared to all other substrates.

With the exception of woodshavings and straw pellets, the use of the remaining substrates changed between weeks 3 and 6 of the cycle (key behaviours affected are illustrated in Figure 2). In the peat rings, there was an increase in the percentage of birds using the peat for dustbathing (U = 36, r = 0.83, P = 0.002), and a reduction in foraging (U = 21, r = -0.83, P = 0.002) and locomotion (U = 1, r = -0.79 P = 0.004) which was parallel to an increase in inactivity (U = 36, r = 0.83 P = 0.002). Similarly, in oat hull rings, an increasing percentage of birds used the rings for dustbathing between weeks 3 and 6 (U = 32, r = 0.65, P = 0.026), and there was a reduction in foraging behaviour recorded (U = 4, r = -0.65, P = 0.026). For the control rings, levels of dustbathing remained consistently low, and levels of sitting inactive remained consistently high. However, the use of the control rings for foraging (U = 0, r = -0.86, P = 0.002), sitting pecking (U = 5, r = -0.60, P = 0.041) and locomotion (U = 0, r = -0.83, P = 0.002) decreased between weeks 3 and 6.

Table 3. The mean number of broilers counted in each substrate each week throughout the production cycle and overall

	Substrate					
	Peat (CI)	Oat hulls (CI)	Straw pellets (CI)	Woodshavings (CI)	Control (CI)	*P*-value
Week 3	17.67 (7.53,26.19)	14.41 (10.10,19.53)	18.18 (10.37,28.16)	29.06 (17.65,43.30)	16.65 (15.78,22.30)	0.05
Week 4	18.88[a] (14.21,24.22)	9.66[b] (5.78,15.54)	12.99[ab] (9.75,16.70)	16.00[ab] (14.42,17.60)	11.21[b] (6.91,15.60)	0.004*
Week 5	20.53[a] (16.76,24.68)	11.15[b] (8.71,13.90)	11.51[b] (9.29,13.97)	18.78[ac] (15.91,21.88)	13.86[bc] (13.20,16.77)	<0.001**
Week 6	12.56[a] (11.47,13.71)	5.58[b] (2.92,9.09)	6.11[b] (2.16, 12.06)	8.42[ab] (6.05,11.18)	7.59[ab] (6.51,9.38)	0.013*
Overall	17.27[a] (15.08,19.61)	9.94[b] (8.02,12.07)	11.78[b] (9.18,14.72)	17.27[a] (13.68,21.28)	12.09[ab] (9.93,14.81)	<0.001*

**P* < 0.05; ***P* < 0.001.;

[a,b,c]Values within a row with different superscripts differ at *P* < 0.05. Means and confidence intervals (CI) have been backtransformed to their original scale.

Table 4 The average number and percentage of broiler chickens observed in each behaviour category in different substrates

Behaviours	Substrate					*P*-value
	Peat (CI)	Oat Hulls (CI)	Straw Pellets (CI)	Woodshavings (CI)	Control (CI)	
Dustbathing						
Mean number of birds[1]	4.01^{a} (2.67, 5.65)	1.40^{b} (0.94, 1.95)	0.07^{c} (0.01, 0.19)	0.02^{c} (0.0019, 0.054)	0.10^{c} (0.00064, 0.054)	<0.001
% of total birds[2]	27.83	18.69	1.79	0.49	0.72	
Foraging						
Mean number of birds	4.23^{a} (2.60, 6.26)	2.70^{a} (1.74, 3.88)	0.36^{b} (0.17, 0.62)	2.60^{a} (1.48, 4.06)	0.16^{b} (0.030, 0.40)	<0.001
% of total birds	28.38	27.16	4.15	17.21	2.56	
Sitting pecking						
Mean number of birds	1.92^{b} (1.44, 2.48)	1.90^{b} (1.43, 2.45)	1.67^{bc} (1.21, 2.21)	4.64^{a} (3.49, 5.95)	0.81^{c} (0.41, 1.35)	<0.001
% of total birds	11.73	21.10	18.84	29.35	7.99	
Sitting inactive						
Mean number of birds	2.47^{b} (1.59, 3.55)	1.72^{b} (1.26, 2.25)	6.71^{a} (4.91, 8.78)	5.74^{a} (3.75, 8.14)	7.72^{a} (6.27, 9.32)	<0.001
% of total birds	17.30	19.37	55.72	37.55	65.40	
Preening						
Mean number of birds	0.44^{ab} (0.22, 0.74)	0.20^{b} (0.095, 0.34)	0.91^{a} (0.61, 1.16)	0.86^{a} (0.61, 1.16)	0.92^{a} (0.70, 1.17)	<0.001
% of total birds	3.95	2.38	8.35	6.05	8.51	

[1]Means and confidence intervals (CI) have been back-transformed to their original scale

[2]For interpretation: values are the percentage of birds performing each behaviour in relation to the average number of birds recorded in the substrate

[a,b,c]Values within a row with different superscripts differ at $P < 0.05$

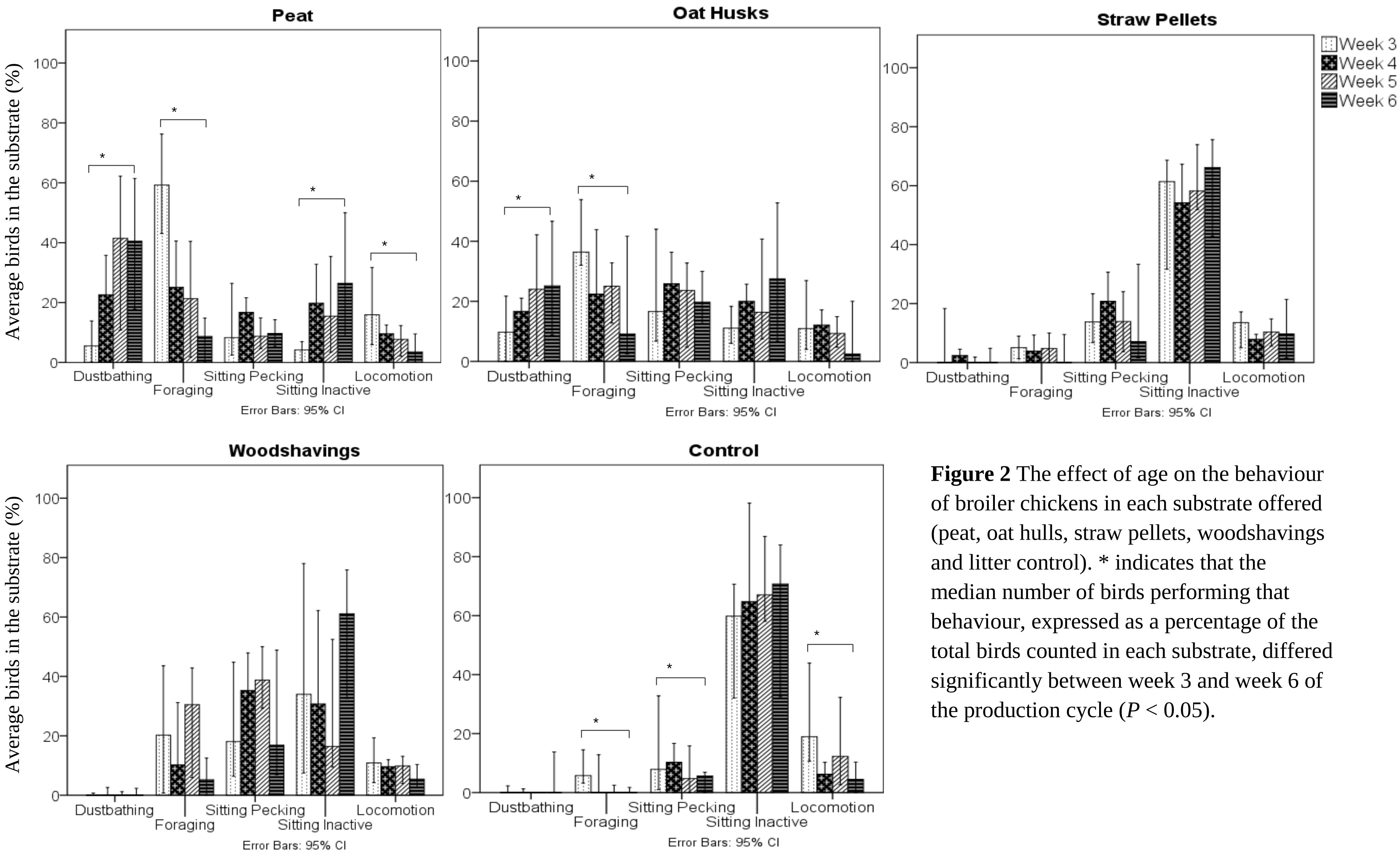

Figure 2 The effect of age on the behaviour of broiler chickens in each substrate offered (peat, oat hulls, straw pellets, woodshavings and litter control). * indicates that the median number of birds performing that behaviour, expressed as a percentage of the total birds counted in each substrate, differed significantly between week 3 and week 6 of the production cycle ($P < 0.05$).

2.3.3 Ring location

There were no significant interactions between location and substrate for ring occupancy ($F_{4,109} = 0.24$, $P = 0.92$), however significantly more birds overall were counted in the central (M = 16.48) compared to the edge rings (M = 12.36; $F_{1,109} = 11.59$, $P = 0.001$). There were no location by substrate interactions for behaviours performed ($P > 0.05$), and no main effect of location on any behaviours ($P > 0.2$).

2.3.4 Dustbathing complexity

There were no significant differences in length of bout or any of the components of a bout between the peat and oat hulls rings (Table 5). There was also no significant effect of substrate on method of bout termination, $\chi^2(1) = 0.105$, $P = 0.75$.

Table 5. Comparison of dustbathing bouts performed by commercial broiler chickens in peat and oat hulls

	Substrate					
	Peat		Oat hulls			
	n	Mean	n	Mean	SEM	*P*-value
Bout length (mins)	24	16.40	24	13.85	0.85	0.13
Number of vertical wingshakes	24	26.38	24	23.00	1.14	0.14
Number of ground pecks	24	179.13	24	205.08	15.49	0.41
Number of leg scratches	24	35.67	24	37.83	2.49	0.67
Number of side rubs	24	27.79	24	24.58	1.98	0.43

2.4 Discussion

All substrates were used by the broilers throughout the cycle, and there were clear distinctions in the types of behaviours performed in each. Although there was no difference in the overall number of broilers counted in each substrate compared to the control, more birds were recorded in the peat and woodshavings rings compared to the oat hulls and straw pellets. Peat was predicted to attract a high number of broilers based on previous work involving laying hens, however this appears to be the first study to demonstrate that broilers show a preference for peat as a dustbathing material. The preference for woodshavings over the more friable and "sand-like" oat hulls was less expected. It may be that some quality of woodshavings makes it an attractive substrate, but the preference may also be influenced by previous experience (Sanotra et al., 1995; Nicol et al., 2001). Although the head count in each substrate gave a general indication of attractiveness, the suitability of substrates as enrichments depends on the types of behaviours they promote. Consistent with previous trials (Petherick and Duncan, 1989), the highest level of dustbathing was seen in peat. Birds also appeared to identify oat hulls as a dustbathing substrate, with significantly more dustbathing performed in oat hulls compared to the remaining substrates. Despite the broilers early experience of woodshavings bedding, the low level of dustbathing observed in the woodshavings rings is consistent with research that showed that birds have an innate ability to identify 'dust' rather than developing a preference based on initial exposure to substrates (Wichman and Keeling, 2008). However, woodshavings did prove to be an attractive foraging substrate, with similarly high levels of foraging performed in peat, oat hulls and woodshavings rings compared to straw pellets and the control. This is consistent with previous trials that have found woodshavings to be attractive for ground scratching and pecking (Petherick and Duncan, 1989; Toghyani et al., 2010). Foraging is a much-reduced behaviour in broiler chickens compared to their ancestors and laying hen counterparts. Modern broilers have been selected for rapid growth rate and increased muscle mass which has resulted in an inefficient, tiring gait pattern (Corr et al., 2003) and a susceptibility to skeletal disorders and deformities that are assumed to be painful (Vestergaard and Sanotra, 1999; Danbury et al., 2000). However, broilers are capable of moving more than they choose to (Reiter and Bessei, 1995; Bessei, 2006), and providing a substrate that promotes foraging would be central in increasing overall activity levels. It is worth noting that although levels of foraging by birds did not differ significantly between woodshavings, peat and oat hulls in the current experiment, levels of sitting inactive were significantly higher in woodshavings. High levels of resting could indicate comfort, however a key aim of providing enrichments for broiler chickens is to reduce the amount of time spent sitting down and encouraging exercise in young broilers, which allows for proper bone and muscle development and improves leg condition (Thorp and Duff, 1988; Reiter and Bessei, 1995).

Broilers physiology and behaviour patterns change significantly over the 6 week cycle, with inactivity increasing to around 80% by slaughter weight (Weeks et al., 2000). Effective enrichments should therefore continue to promote activity as birds age. In this trial, there was an expected decrease in foraging behaviour in older birds, however there was an increase in the percentage of broilers using preferred substrates for dustbathing. Current literature is inconsistent on the effect of age on dustbathing behaviour in domestic fowl, with reports of no effect of age (Weeks et al., 2000; Cornetto and Estevez, 2001b; Shields et al., 2004; Bailie et al., 2013; Villagrá et al., 2014), and some trends of increased dustbathing to peaks at around week 6-7 (Weeks et al., 1994; Bokkers and Koene, 2003). These increases in dustbathing may be consistent with the normal development of the behaviour. In red junglefowl, dustbathing frequency and vertical wingshakes increase in young birds until it stabilises at around 3-4 weeks (Hogan et al., 1991). They may also, however, reflect an increased redirection of the behaviour towards more suitable substrates as house litter quality declines. There was no apparent increase in dustbathing in the straw pellet, wood shaving and control rings which may suggest that the lack of age effect noted in some previous studies was due to a lack of suitable substrate. The percentage of birds foraging declined with age in peat, oat hull and control rings, and remained low throughout in straw pellets. Once birds get larger and their gaits become more inefficient (Corr et al., 2003), energy resources are likely to be reallocated and the reduction in foraging can be explained as an adaptive reduction in contrafreeloading (Lindqvist et al., 2006). Dustbathing behaviour is likely to be less affected by this phenomenon and the motivation for dustbathing may remain higher.

More precise measures of the components of dustbathing performed in the peat and oat hulls rings were used to investigate whether one substrate was more capable of satisfying the motivation than the other. No significant difference was found in bout length, method of termination, number of vertical wingshakes or any other elements. Given the overall higher attractiveness of peat, a difference in dustbathing structure may have been expected. Vestergaard et al. (1990) recorded very little difference in the frequency and components of dustbathing in jungle fowl birds housed on either wire or sand. However, they did find that dustbathing bouts tended to be longer on wire and that in longer bouts birds were more likely to end the dustbathing with a bodyshake in sand compared to wire. They propose that although dust may not be required to begin a dustbathing bout, hence sham dustbathing, it may be important in giving the feedback that ends the bout. This would suggest that although the lack of difference in components cannot necessarily mean that peat and oat hulls were an equally satisfying “dust”, the lack of difference in how the bout was terminated could show that they were both providing the necessary feedback of a proper dustbathing substrate. However, Petherick and Duncan (1989) found that hens dustbathe in peat for significantly longer than in sand, sawdust and woodshavings, which they interpret as meaning that peat is more satisfying and preferred. This infers that oat hulls and peat may be considered equally satisfactory as a dustbathing substrate.

The location of the rings (either edge or central) did not have an effect on the types of behaviours performed. However, overall there were more birds counted in the rings in central areas of the house which was unexpected as broilers have a tendency to stay near pen walls (Cornetto and Estevez, 2001a). The edge rings in this trial were not located against the house walls, which means birds crowding directly against the walls were unlikely to come into contact with the rings, reducing the edge effect expected. Litter moisture is considered to have multidimensional causal factors and varies between farms, house design and cycle, however in this house it was noted that litter tended to be wetter towards the edges, which could also account for increased occupancy in the central areas.

Dustbathing is considered to be a highly-motivated behaviour, however there is limited information on the overall level of dustbathing performed in commercial settings, with dustbathing sometimes excluded from broilers ethogram or not observed at all throughout the trial (e.g. Murphy and Preston, 1988). However, the consensus is that dustbathing makes up a very small portion of broilers time budget, with reports of the % of birds dustbathing over the cycle averaging at 0.38% (Thomas et al., 2011), 0.57% (Weeks et al.,1994) and 0.18% (Bailie et al., 2013) in birds housed on woodshavings, and 1% (Shields et al., 2004) with constant access to sand. The average proportion of birds using the rings for dustbathing in this trial was substantially higher in some cases; the average % of birds dustbathing in rings over the whole cycle was 28% in peat, 19% in oat hulls, 2% in straw pellets, 0.5% in wood shavings and 0.7% in the control treatment. The control rings were undisturbed throughout the cycle and represented the litter quality around the rest of the house. In later weeks, litter becomes increasingly mixed with faeces and feed which creates a more compacted and wet material, making it unsuitable for dustbathing. The low levels of dustbathing seen in the control treatment are therefore likely to represent levels of dustbathing seen in broilers sheds with similar bedding. However, overall 10% of broilers using the novel substrates offered were observed dustbathing. This suggests that an appropriate dustbathing substrate may stimulate a higher level of dustbathing than would normally be observed in a commercial house.

2.5 Conclusions

In conclusion, our findings are consistent with previous laying hen research that indicates peat is an attractive substrate to domestic fowl and promotes high levels of dustbathing. Further work would be useful to determine the nature of the qualities that make peat attractive. As peat is considered an impractical addition to UK farming systems, oat hulls may be suitable as an alternative commercial enrichment. In the present experiment, oat hulls stimulated significantly more dustbathing than straw pellets, woodshavings or litter, and promoted similarly high levels of foraging and low levels of inactivity compared to peat. There was no difference in the duration or components of dustbathing bouts performed in peat and oat hulls, suggesting they both satisfy the broilers motivation to dustbathe. One limitation to the use of oat hulls, which was not measured in the current study but which should be considered in subsequent research, is its effect on dust levels within the house. The clear change in proportional use of the peat and oat hulls, with an increase in dustbathing and reduction in foraging over time, suggests that dustbathing will continue to be performed as broiler chickens age, and therefore that provision of a suitable dustbathing substrate will provide effective environmental enrichment for commercial broiler chickens throughout the cycle.

Chapter Three

Study 2

Evaluation of a dustbathing substrate and straw bales as environmental enrichments in commercial broiler housing

Preface

Based on the results of the pilot trial described in Chapter 2, oat hulls appeared to be an attractive material and readily identified as a dustbathing substrate by broilers. Therefore, in order to determine whether oat hulls would be an appropriate dustbathing enrichment, a largescale study was designed to run parallel to the main preference experiment in Chapter 2. Several points need to be considered before recommendations can be made for the inclusion of oat hulls as a commercial dustbathing enrichment:

- Are oat hulls successful at stimulating dustbathing behaviour in commercial broiler chickens, and maintaining interest throughout the production cycle?
- Do oat hulls provide broilers with a different stimulation compared to the straw bales already currently provided?
- Are there any benefits of including a dustbathing material on broiler leg health or activity levels?
- Would oat hulls be a replacement for straw bales or a supplementary enrichment?
- Do oat hulls have any effect on production or environmental parameters?
- What are the practicalities involved in using oat hulls on farms? (e.g. method of containing and distributing oat hulls).

Previous research has found that including a relatively high quantity of long-cut straw bales led to an increase in overall broiler activity levels (Kells et al., 2001). However, the protocols for straw bale inclusion for this producer differed significantly from that research. There was also therefore an interest in knowing whether plastic wrapped straw bales, supplied at the density typically outlined by this producer, was producing a similar effect on activity levels and broiler behaviour.

Abstract

The use of straw bales as an environmental enrichment is common for broiler chickens in enriched housing systems, however relatively little information exists about their effectiveness in improving welfare. There has also been no widespread introduction of a dustbathing material for broilers. The main aim of this trial was to

evaluate the use of a dustbathing substrate (in the form of oat hulls), both as an alternative to straw bales and as a supplementary enrichment. Over four replicates, four commercial houses, each containing approximately 22 000 broilers, were assigned to one of four treatments over the 6 week production cycle: (1) straw bales (B; one per 155 m^2), (2) oat hulls as a dustbathing substrate (OH; provided in 1 m diameter steel rings, one per 155 m^2), (3) both oat hulls and straw bales (OH+B), and (4) a control treatment with no environmental enrichment (C). Observations of broiler behaviour and leg health were taken weekly, and performance data was collected for each cycle. Broilers housed in the OH and OH+B treatments had better gait scores in week 6 than those housed in the C treatment ($P < 0.05$), which suggests that the provision of oat hulls improved bird leg health. However, there was no associated increase in activity levels in unenriched areas of the houses. Conversely, more locomotion ($P < 0.001$), less sitting inactive ($P < 0.001$) and less sitting pecking ($P < 0.001$) were observed in the C treatment than in unenriched areas of B, OH and OH+B treatments. More birds were recorded around the bales compared to the oat hulls ($P < 0.001$), however birds performed significantly more foraging ($P = 0.019$) and dustbathing ($P = 0.045$) in oat hulls than around straw bales. Although oat hulls appear to be more suitable for stimulating active behaviours than straw bales, the high level of resting recorded around the bales suggests they may have a positive function as protective cover. The presence of an additional type of enrichment in the house did not affect the number of birds, or the type of behaviours performed in close proximity to either straw bales or oat hulls ($P > 0.05$). Treatment did not have a significant effect on pododermatitis levels or slaughter weight, on mortality rates, or on litter quality or atmospheric ammonia levels ($P > 0.05$). Overall, our results suggest that the oat hulls substrate was a successful enrichment in terms of promoting dustbathing and foraging, and improving bird leg health. The straw bales also appeared attractive to the birds, however, which suggests that a dustbathing substrate should be a supplementary enrichment.

3.1 Introduction

Broiler chickens are typically housed in indoor systems, in groups of several thousand, and bedded on deep litter. With the exception of feeder and drinker lines, the houses do not usually contain additional furniture or stimulation. Providing domestic fowl with more complex environments has improved stereotypical pecking behaviours (Nørgaard-Nielsen et al., 1993), fear reactions (Jones and Waddington, 1992; Reed et al., 1993), learning (Krause et al., 2006), activity levels (Kells et al., 2001) and leg condition (Mench et al., 2001; Bizeray et al., 2002a). Chickens will readily enter areas containing novel items (Newberry, 1999) and will spend more time in preferred foraging and dustbathing substrates when provided (Shields et al., 2004). Crucially, introducing barriers (Bizeray et al., 2002a) and straw bales (Kells et al., 2001) has been shown to increase activity levels in broilers. Modern broilers will spend up to 86% of their time sitting down (Weeks et al., 2000), with this inactivity linked to a high prevalence of skeletal conditions and leg disorders that get worse with age (Vestergaard and Sanotra, 1999; Danbury et al., 2000; Knowles et al., 2008). Providing broilers with a more complex environment is therefore likely to improve bird welfare, both by improving leg health and by providing a stimulating environment to promote natural behaviours (Newberry, 1995).

Although there is no current legal requirement for broilers to be provided with environmental enrichment, those housed under conditions dictated by welfare assurance schemes are often supplied with some variation of natural light, perches and/or straw bales (e.g. CIWF, 2017). Foraging and dustbathing are highly motivated behaviours and preventing birds from performing them leads to observable frustration (Lindberg and Nicol, 1997; Vestergaard et al., 1997; Fraser and Duncan, 1998). Providing a foraging substrate, in the form of straw bales, should therefore have a positive effect on welfare. However, there is limited research on the use of bales provided at a commercial level. Kells et al. (2001) showed that providing broilers with straw bales increased their overall activity levels, however their trial used a higher number of bales than are supplied commercially. More recent research that involved lower straw bale densities, chosen to more closely reflect current industry practice, did not yield similar findings (Bailie et al., 2013; Bailie and O’Connell, 2014). Similarly, although smaller scale research has been conducted on the preference of broilers for different dustbathing substrates (e.g. Shields et al., 2004), there has been no research on the provision of dustbathing enrichments in commercial housing. Dustbathing consists of birds kicking a loose friable substrate through their feathers and is a highly-motivated behaviour (van Liere et al., 1991; Vestergaard et al., 1997; Vestergaard and Sanotra, 1999). Broilers with tibial dyschondroplasia will dustbathe significantly less than their healthy counterparts, which may be due to dustbathing requiring rotation and movement of the legs (Vestergaard and Sanotra, 1999). Domestic fowl have shown a preference for peat and sand as dustbathing materials (Shields et al., 2004; de Jong et al., 2007), however these substrates are expensive, unsustainable and may interfere with the litter removal process. A practical alternative has been suggested in the form of ground oat hulls, which are a by-product of oat milling, however their effectiveness as a form of environmental enrichment has not yet been evaluated under commercial conditions.

This experiment was designed to evaluate different environmental enrichment conditions for commercial broiler chickens. This included assessing the effectiveness of straw bales (when provided at a level that reflects practice on some commercial farms), a comparable quantity of oat hulls, both straw bales and oat hulls, and a control treatment with no straw bales or oat hulls. There was a particular interest in understanding whether oat hull dust baths should be used as an alternative or supplementary form of environmental enrichment to straw bales. The effects of different enrichment treatments on general behaviour of the birds (both in close proximity to, and away from the enrichments), on measures of health and performance, and on environmental measures within the house were determined.

3.2 Materials and methods

3.2.1 Subjects and housing

A total of 355 400 Ross 308 broiler chickens (Aviagen Ltd, UK) were used in this study and were reared from a day old on a commercial farm in Northern Ireland. The trial was repeated for four production cycles between July and December 2015. Four metal framed, windowed broiler houses were used on this farm. Two houses had a floor space of 1 398 m^2 and two had a floor space of 1 395 m^2 due to different positioning of outbuildings. Approximately 22 000 birds were placed in each house ‘as hatched’, which gave an approximate 50:50 mix of males and females. This gave an initial stocking density of 16 birds /m^2. A proportion of the birds were removed for thinning at approximately day 30, and the remaining birds were cleared between days 37 and 42. House temperature, humidity and light levels were controlled in the same manner as described in Chapter 2 (2.2.1).

3.2.2 Treatments and experimental design

In order to investigate the effectiveness of different enriched conditions, the four commercial houses were assigned to one of four treatments: 1) Bales (B), 2) Oat Hulls (OH), 3) Oat Hulls and Bales (OH+B), 4) Control (C). This trial was repeated over four cycles, with each house assigned to each treatment once. No environmental enrichment was provided in the control treatment. In treatments containing straw bales, bales were piled on top of one another around the edges of the commercial house, as was standard practice on this farm. On day 10, nine plastic-wrapped bales of chopped straw (approximately 0.8 m long x 0.4 m wide x 0.4 m high) were placed evenly around the house, which again matched normal practice on this farm. As discussed in the methodology of Chapter 2, the introduction of substrates on day 10 was chosen for practical reasons. Five bales were placed down the central line of the house and four around the edge of the house. The sides of the plastic bales were cut open to allow access to the straw (Photo 2), and once the top of the bale had collapsed through use, it was replaced in the same location. Existing bales were dismantled (and plastic removed) just prior to thinning, and were replaced with nine new bales after thinning. In total, two bales per 1 000 birds (46 bales; 1 per 155 m^2) were used across a 6 week cycle in a particular house.

Photo 2. Photograph of the enrichments used throughout the trial: plastic-wrapped, short cut straw bales (left) and steel rings of ground oat hulls (right) An example of the tripod and camera position can be seen next to the oat hulls (right).

Oat hulls are the ground outer hull of oats, produced as a by-product of oat milling (Whites Speedicook Ltd, Craigavon, UK), with a colour and consistency similar to fine sawdust. Oat hulls have previously been used in nutritional trials with broilers chickens (e.g. Hetland and Svihus, 2001) and were considered a safe substrate to introduce to broilers. The oat hulls were sourced locally and delivered in 1 tonne bags, which were placed in a central area of the OH and OH+B houses before the birds were placed. This reduced the floor space available by approximately 4 m^2. Small groups of broilers were observed grouping around the bags but their presence was not expected to account for any significant variation in the results. Oat hulls were distributed to rings using buckets filled from these central bags. There has been some discussion about potential alternative storing methods for oat hulls, for example baling (Moy Park Ltd, personal communication). It would be important to develop a simple way of storing and distributing oat hulls that minimised labour.

The oat hulls were provided in a manner which attempted to emulate the level of provision of straw bales. Nine stainless steel rings (1 per 155 m^2; 1.1 m diameter, 7.6 cm deep) were placed in corresponding sections of the house to the B treatment. The area of the rings (~0.95 m^2) was chosen such that it was equal to the area of two sides and two ends of a straw bale. The rings were placed in the house on day 10 and filled with approximately 9 kg of oat hulls. Oat hull rings were then topped up to the original level throughout the cycle when more than half of the substrate in them had gone. Oat hulls were always topped up to their original condition on the morning of observations to ensure they were in a standardised condition. In the OH+B treatment both types of enrichment were placed in the corresponding sections of the house that contained enrichments in the other treatments; there was always a feeder or drinker line separating the two enrichments which were placed approximately 1.5 m apart.

The steel rings used in this trial were deemed the most practical way of creating a contained dustbathing area while preventing broilers from perching and obstructing access to the dust bath. Initial designs for dust baths were wooden squares, however these were more easily perched upon, broke down in the damp and humid conditions, and could not be cleaned between cycles. Steel rings were pressure washed by the farmer hosting this trial between production cycles to maintain biosecurity. This may be impractical and time-consuming to implement commercially. It would be useful to investigate alternative methods of containment, or indeed whether oat hulls placed in cleared areas of the floor would create a suitable dustbathing area.

3.2.3 Data Collection

The farm was visited twice a week in weeks 3, 4, 5 and 6 of each cycle and all measurements were taken by the same observer.

Video recordings of broiler behaviour were performed on the first day of data collection each week using five Toshiba Camileo X-Sports cameras placed on 1.5 m high wooden tripods (Photo 2). Using feeder and drinker lines, the house was virtually sectioned into 66 approximately equal sections. These sections were classified as “enriched” (sections that contained an enrichment), “unenriched”, “edge” (sections that had a side made up of the house wall) and “central”. In each house, a total of four hours of video footage was taken between 9:00 h and 15:00 h per week. This consisted of half-hour recordings taken in eight different locations. In the B and OH treatments, four half-hour recordings were taken of four randomly chosen enrichments, two central and two edge. The remaining four half-hour videos were taken of unenriched areas of the house, two in edge locations and two in central locations. In the C treatment, sections chosen corresponded to “enriched” and “unenriched” sections of the other treatments. In the B+OH treatment, the same approach was adopted as above except that a second camera was used to allow for both types of enrichment to be recorded in the enriched sections of the house. The cameras were set up in all four houses and were switched on one after another by the same observer; the order that the cameras were switched on was therefore randomised to control for the slight difference in video starting times.

To analyse footage, for each half hour video (n total = 512) scan sampling was used to count the number of birds and to categorise the behaviour of each bird according to a simplified version of the ethogram used in Chapter 2 (Table 6). The % of birds engaged in different behaviours was then calculated. Two scans were performed per recording, one at 10 minutes and one at 20 minutes. The “scan areas” were balanced as far as possible considering the different enrichments filmed. In footage containing a ring, all birds inside the rings were counted and categorised. In bale videos, a side and end of the bale were outlined and transposed onto the floor area around the bale, which gave an area equivalent to half the area of a ring and equated to approximately 0.4 m in front of and to the side of the bale. As only one side of the bale could be filmed, this count was doubled for analysis, as in Kells et al. (2001). In footage of empty (unenriched) floor area, an outline of a ring was used in the centre of frame and birds with more than half their body across this line were counted and categorised.

Table 6. Ethogram of broiler chicken behaviours used in the present trial, based on Cornetto and Estevez (2001b) and Shields et al. (2005)

Behaviour	*Definition*
Dustbathing	Birds were performing classic vertical wing shakes, and/or clearly covered in substrate and performing side-rubs or prone leg scratches
Foraging	Scratching and pecking at the substrate (from a standing or walking position)
Sitting inactive	Sitting with no other activity
Sitting pecking	Sitting and ground pecking
Locomotion	Standing or walking, with no other pecking or scratching activity
Sitting preening	Preening, running beak through feathers, while sitting
Standing preening	Preening, running beak through feathers, while standing
Resting	Sitting with head under wing, eyes obviously closed, or lying on one side with a leg and/or wing stretched out
Other	Any other behaviours

On the second day of data collection each week, environmental measures and gait scores were recorded. Litter samples were taken from eight random locations around the house, four from central sections and four from edge sections. Samples were collected in plastic bags, thoroughly mixed and stored in a cool box for transport. Following drying for 24 hours at 70^{o}C, the dry matter percentage of the litter was calculated (McLean et al., 2002; Bailie et al., 2013). To give an indication of ammonia within each house, pHydrion^{TM} (Dewey et al., 2000) paper tests were used in four locations (two front and two back) in each house. Each test strip was moistened with distilled water and held at bird head height for 15 seconds, after which the colour of the paper gave an indication that ammonia was either 0, 5, 10, 20, 50 or 100 ppm. Broilers houses are legally required to not exceed ammonia concentrations of 20 ppm. These four scores were averaged to give an average ammonia score per house, per week. Gait scoring was performed using the Modified Gait Scoring Method (Garner et al., 2002). Each week, two birds were gait scored from 20 random sections of each house. Within the sections, the two birds were randomly chosen using a numbered grid on a perspex sheet (Kells et al., 2001; Bailie et al., 2013). The sheet was held at arms length and the birds closest to the randomly generated co-ordinates on the grid were given a gait score of 0-5 (Garner et al., 2002).

Mortality (which is the number of birds removed dead from the house and does not include culled birds), downgrades (which consists of birds deemed imperfect at the slaughterhouse, for example due to contamination, damage at defeathering or being undersized), the number of culls performed and slaughter weight of the birds were taken from company records. Levels of pododermatitis were recorded at slaughter in one hundred birds per house at thinning and one hundred birds per house at final clearing. Pododermatitis was recorded by slaughterhouse staff on a scale of 0-2, where '0' represents either no pododermatitis or very superficial lesions, '1' represents mild pododermatitis on either foot with discolouration of the footpad and superficial lesions, and '2' is recorded when there is severe pododermatitis on either foot with ulcers, signs of haemorrhage and/or swollen footpads.

3.2.4 *Statistical analysis*

All data were analysed using IBM SPSS Statistics (Version 23). Data normality was assessed through inspection of residual histograms, Q-Q plots and Shapiro-Wilk tests. Where equal variance could not be assumed, adjusted degrees of freedom are presented. Post-hoc tests, where applied, were chosen based on whether the assumptions of equal variance and equal sample size were met.

Scan data representing the number of birds close to each type of enrichment (i.e. in oat hull rings or close to straw bales), and the percentage of birds engaged in different behaviours while close to each type of enrichment and while in unenriched areas, were averaged within-treatment each week. Data on the % of birds engaged in different behaviours in unenriched areas could not be sufficiently transformed for parametric analysis. Therefore, the main effects of "treatment" (OH, B, OH+B, C) and of "age" were analysed using Kruskall-Wallis tests. The main effect of "cycle" was also tested and no significant effects were found for any behaviour ($P > 0.05$). Dustbathing and Other were infrequently recorded and were excluded from analysis.

To determine whether the presence of nearby straw bales or oat hulls had an effect on the way individual enrichments were used, oat hulls and bales in the single treatments

(OH, B) were compared with their counterparts in the OH+B treatment. The total number of birds and occurrence of each behaviour (%) in the rings of oat hulls in the OH compared to the OH+B, and around the bales in the B compared to OH+B treatment were analysed. Independent samples t-tests were used to compare total numbers of birds. GLMM was used to compare the percentage of birds observed in each behaviour category between the single and combined treatment, with “treatment” and “age” as fixed factors and “cycle” as a random factor; a log10 transformation and +1 constant was applied to improve normality where necessary.

To compare the use of oat hulls and straw bales in general, data from enrichments in single treatments (OH or B) were combined with their counterpart in the combined treatment (OH+B) to give combined data for oat hulls (from OH and OH+B) and for bales (from B and OH+B). An independent samples t-test was used to compare the combined total number of birds interacting with the straw bales and oat hulls. The difference in behaviours (%) performed in oat hulls and bales was compared using GLMM. Each behaviour was modelled separately, with “enrichment type (OH or B)” and “age” as fixed factors and “cycle” as a random factor. Significant interactions were further investigated using simple effects analysis. Where there was a significant main effect, post-hoc tests were performed using a Tukey test where equal variance could be assumed and a Games-Howell test when this assumption was violated. Preening was infrequently recorded, and therefore “standing preening” and “sitting preening” were grouped to facilitate analysis.

Performance data and levels of pododermatitis were recorded once at the end of each cycle, and, as such, the GLMM for analysis consisted of “treatment” as a fixed factor and “cycle” as a random factor. Gait score data were ordinal and the effect of treatment was analysed using Kruskall-Wallis tests within weeks, with follow-up stepwise stepdown multiple comparison (based on Campbell and Skillings, 1985). Ammonia measures were analysed using a one-way ANOVA to compare average ammonia between treatments (OH, B, OH+B, C). Litter moisture data were analysed using GLMM with “treatment” and “age” as fixed factors and “cycle” as a random factor.

3.3 Results

Over the four production cycles, a total of 8876 broilers were observed and categorised according to Table 6 in unenriched areas of the houses, 3779 broilers were observed around one side of the bales, and 3729 broilers inside the oat hulls.

3.3.1 Behaviour in unenriched areas of all treatments

Treatment had a significant effect on the majority of the behaviours observed (median values presented in Table 7). Birds in the control treatment performed less sitting inactive (H(3) = 36.8, n = 64, P < 0.001) and sitting pecking (H(3) = 35.5, n = 64, P < 0.001), and more locomotor behaviour (H(3) = 36.6, n = 64, P < 0.001) compared to birds in the three enriched treatments. Higher levels of preening while birds were sitting down was observed in the OH+B compared to the control treatment (H(3) = 7.9, n = 64, P = 0.048), and significantly more preening while standing was performed in the control compared to the enriched treatments (H(3) = 24.3, n = 64, P < 0.001). There were no differences in the levels of foraging or resting observed between treatments (P > 0.05). Foraging was the only behaviour to be significantly affected by age (H(3) = 22.78, n = 64, P < 0.001); the median percentage of birds foraging was 2.5 in week 3, 1.0 in week 4, and 0 in weeks 5 and 6. Pairwise comparisons showed that foraging was significantly higher in week 3 compared to week 5 (P < 0.001) and week 6 (P = 0.001).

3.3.2 Effect of the presence of an alternative enrichment

There was no significant difference in the mean number of birds recorded in the rings in the OH (M = 14.49, SE = 1.44) compared to the OH+B (M = 15.05, SE = 1.68) treatment (t(30) = -0.25, P = 0.80), or the mean number of birds in close proximity to the bales in the B (M = 28.69, SE = 2.91) compared to the OH+B (M = 30.91, SE = 3.15) treatment (t(30) = -0.52, P = 0.61). There were also no differences in the level of any behaviours in the single compared to the combined treatments (Table 8) and no significant interactions.

Table 7. The effect of enrichment type on the behaviour of broilers in unenriched areas of the house, on health and productivity measures, and on environmental measures

	Treatment				
	Oat Hulls	Bales	Oat Hulls + Bales	Control	*P* value
Behaviour in unenriched areas[1] (%):					
Foraging	0.99 (0.00, 2.12)	1.03 (0.00, 2.42)	0.00 (0.00, 1.84)	0.77 (0.00, 2.24)	ns
Sitting Inactive	54.82 (52.81, 63.37)[a]	54.46 (49.05, 59.27)[a]	54.52 (45.44, 59.04)[a]	7.22 (4.17, 11.29)[b]	<0.001
Sitting Pecking	7.57 (3.96, 10.64)[a]	8.66 (6.82, 10.60)[a]	6.00 (5.01, 9.68)[a]	0.00 (0.00, 0.00)[b]	<0.001
Locomotion	9.04 (7.14, 10.89)[b]	12.44 (6.19, 14.01)[b]	9.30 (3.36, 14.10)[b]	61.48 (56.77, 67.16)[a]	<0.001
Sitting Preening	7.09 (4.51, 9.52)[ab]	6.56 (4.78, 8.42)[ab]	9.52 (7.13, 11.06)[a]	5.64 (4.80, 6.31)[b]	0.048
Standing Preening	0.00 (0.00, 1.20)[b]	0.84 (0.00, 1.71)[b]	0.87 (0.52, 2.04)[b]	7.71 (4.20, 9.57)[a]	<0.001
Resting	6.68 (5.23, 13.44)	12.60 (4.55, 16.22)	15.47 (8.05, 23.48)	10.98 (5.11, 16.16)	ns
Health and performance[2]:					
Pododermatitis (%)	33.64 ± 10.45	35.18 ± 10.70	29.19 ± 11.84	33.06 ± 8.43	ns
Average slaughter weight (g)	2.10 ± 0.047	2.12 ± 0.05	2.17 ± 0.05	2.19 ± 0.39	ns
Mortality (%)	1.55 ± 0.09	1.08 ± 0.12	1.04 ± 0.16	1.32 ± 0.15	0.003
Culls (%)	0.63 ± 0.17	0.41 ± 0.09	0.53 ± 0.14	0.34 ± 0.043	ns
Downgrades (%)	0.71 ± 0.17	0.70 ± 0.07	0.70 ± 0.13	0.61 ± 0.075	ns
Environmental measures[2]:					
Litter moisture (%)	26.08 ± 2.92	28.73 ± 2.22	26.46 ± 2.10	28.06 ± 1.75	ns
Ammonia (ppm)	6.46 ± 2.35	7.50 ± 3.24	6.35 ± 3.05	7.60 ± 3.15	ns

[1]Median values (95% confidence intervals); [2]Mean values ± standard error.
Different letters along horizontal rows indicate significance in pairwise comparisons from Kruskall-Wallis rank test.

3.3.3 Differences in the use of oat hulls and straw bales

There was a significant interaction between enrichment type and age for dustbathing ($F_{3.9}$ = 8.004, P = 0.007) and foraging ($F_{3,9}$ = 12.08, P = 0.002) (Table 8), which indicates that birds used the two enrichment types differently as they aged (Figure 3). Specifically, the amount of birds foraging and dustbathing changed over time in the oat hulls but not around the bales. The mean percentage of birds in the oat hulls that were dustbathing increased as birds aged (week 3, M = 5.04%, SE = 1.31; week 4, M = 12.27%, SE = 3.08; week 5, M = 17.61%, SE = 5.29; week 6, M = 21.22%, SE = 2.98), but very few incidences of dustbathing were recorded around the bales throughout the production cycle (week 3, M = 0%; week 4, M = 1.21%, SE = 1.20; week 5, M = 0.12%, SE = 1.16; week 6, M = 0%). More foraging was consistently seen in the oat hulls compared to around the bales, however levels of foraging decreased in the oat hulls over time (week 3, M = 48.42%, SE = 5.99; week 4, M = 24.62%, SE = 3.44; week 5, M = 23.02%, SE = 2.77; week 6, M = 19.01%, SE = 3.38) and remained similar around the bales (week 3, M = 7.00%, SE = 1.26; week 4, M = 6.18%, SE = 2.10; week 5, M = 6.37%, SE = 1.44; week 6, 5.84%, SE = 2.08).

In addition to the interactions described above, significant main treatment effects were also found (Table 9). For example, a higher level of sitting pecking was observed in the oat hulls, while birds around the straw bales showed more inactivity, preening and “other” behaviours. There was also a main effect of age on preening, however no enrichment by age interaction was seen and levels generally varied between weeks (week 3, M = 5.22%, SE = 0.78; week 4, M = 6.54%, SE = 0.80; week 5, M = 3.48%, SE = 0.62; week 6, M = 5.78%, SE = 1.72).

Overall, significantly more birds were recorded around the bales (M = 29.80, SE = 2.12) than inside the oat hulls (M = 14.77, SE = 1.09; t(46.3) = -6.31, P < 0.001).

3.3.4 Health and performance

Treatment did not have a significant effect on average bird slaughter weight, culls, downgrades or levels of pododermatitis *(P* > 0.05) (Table 7). However, there was a significant effect of treatment on % mortality (*P* = 0.003), with post-hoc tests showing a trend for higher levels of mortality in the oat hulls compared to the oat hulls and bales (*P* = 0.070), however there was no significant difference between any of the enriched treatments and the control (OH 1.55%, SE = 0.09; B 1.08%, SE = 0.12; OH+B 1.05%, SE = 0.15; C 1.32%, SE = 0.14; *P* > 0.40). The lack of clear differences between individual treatments for mortality are likely to be due to the impact of cycle within the model as there was an unexplained high level of mortality in one cycle.

3.3.5 Leg health

A total of 2560 birds were gait scored over the four production cycles. The distribution of gait scores between treatments for each week are presented in Table 10. More birds were classified with worse gait scores over time, in all treatments. Treatment had no effect on gait score in weeks 3, 4 and 5 (*P* > 0.05), however there was a significant effect of treatment in the final week (H(3) = 8.19, *P* = 0.042). In week 6, birds provided with oat hulls (mean rank 305.60) or oat hulls and bales (mean rank 304.44) had lower gait scores than birds in the control treatment (mean rank 350.72; *P* < 0.05). Birds in the bales treatment (mean rank 321.24) had similar gait scores to the oat hulls, oat hulls + bales (*P* = 0.57) and control treatments (*P* = 0.79).

3.3.6 Environmental Measures

There was no significant effect of treatment on litter moisture or ammonia levels (*P* > 0.05) (Table 7). Age did have an effect on litter moisture ($F_{3,64}$ = 5.20, *P* = 0.03), with a temporary increase in overall litter moisture in week 4 of the cycle. However, no overall increase was seen over time, with no significant difference between weeks 3, 5 and 6.

Table 8. The behaviour of broilers using enrichments in the single treatment (either OH or B), compared to their counterpart in the combined treatment (OH+B)

	Oat Hulls			**Bales**		
Behaviour (%):	OH	OH+B	*P* value	B	OH+B	*P* value
Dustbathing	12.67 (6.73, 18.60)	15.41 (9.32, 21.50)	0.346	0.058 (-.065, 0.18)	0.60 (-.68, 1.89)	0.391
Foraging	28.39 (18.73, 38.05)	29.15 (21.54, 36.76)	0.876	4.62 (3.07, 8.20)[1]	5.67 (2.83, 8.20)[1]	0.528
Sitting Inactive	17.94 (12.43, 23.46)	16.50 (12.56, 20.45)	0.795	49.87 (41.67, 58.08)	49.03 (39.23, 58.83)	0.818
Sitting Pecking	23.34 (19.0, 28.65)[1]	22.01 (17.88, 27.05)[1]	0.290	10.21 (6.92, 13.50)	8.88 (6.28, 11.49)	0.680
Locomotion	8.38 (5.25, 11.51)	9.81 (7.42, 12.20)	0.176	4.62 (3.07, 6.76)[1]	5.67 (3.83, 6.76)[1]	0.506
Preening	3.78 (2.50, 5.06)	2.74 (1.28, 4.20)	0.279	7.76 (4.77, 10.75)	6.74 (4.62, 8.86)	0.452
Resting	1.74 (0.81, 3.14)[1]	1.97 (0.96, 3.50)[1]	0.445	4.98 (3.12, 7.69)[1]	3.93 (2.40, 6.16)[1]	0.294

[1]Means and confidence intervals have been backtransformed to their original scale

Table 9. The effects of enrichment type and age on behaviours performed in oat hulls and around straw bales

	Mean ± SE		Enrichment		Age		Age*Enrichment	
Behaviour (%):	Oat Hulls	Bales	F (df)	*P* value	F (df)	*P* value	F (df)	*P* value
Dustbathing	14.04 ± 1.98	0.33 ± 0.30	10.98 (1,3)	0.045	6.373 (3,9)	0.013	8.004 (3,9)	0.007
Foraging	28.77 ± 2.84	6.35 ± 0.84	21.66 (1,3)	0.019	11.17 (3,9)	0.002	12.09 (3,9)	0.002
Sitting Inactive	17.22 ± 1.57	49.46 ± 2.95	37.84 (1,3)	0.009	1.24 (3,9)	ns	1.58 (3,9)	ns
Sitting Pecking	24.25 ± 1.56	9.55 ± 0.97	14.97 (1,3)	0.031	1.45 (3,9)	ns	1.97 (3,9)	ns
Locomotion	9.10 ± 0.92	19.95 ± 2.83	9.89 (1,3)	0.051†	0.56 (3,9)	ns	3.88 (3,9)	0.050†
Preening	3.26 ± 0.46	7.25 ± 0.85	11.93 (1,3)	0.041	4.09 (3,9)	0.044	0.58 (3,9)	ns
Resting	3.14 ± 0.89	5.83 ± 0.79	3.75 (1,3)	ns	2.85 (3,9)	0.097†	3.01 (3,9)	0.087†
Other	0.22 ± 0.078	1.31 ± 0.20	12.34 (1,3)	0.039	0.63 (3,9)	ns	0.30 (3,9)	ns

† $P < 0.1$

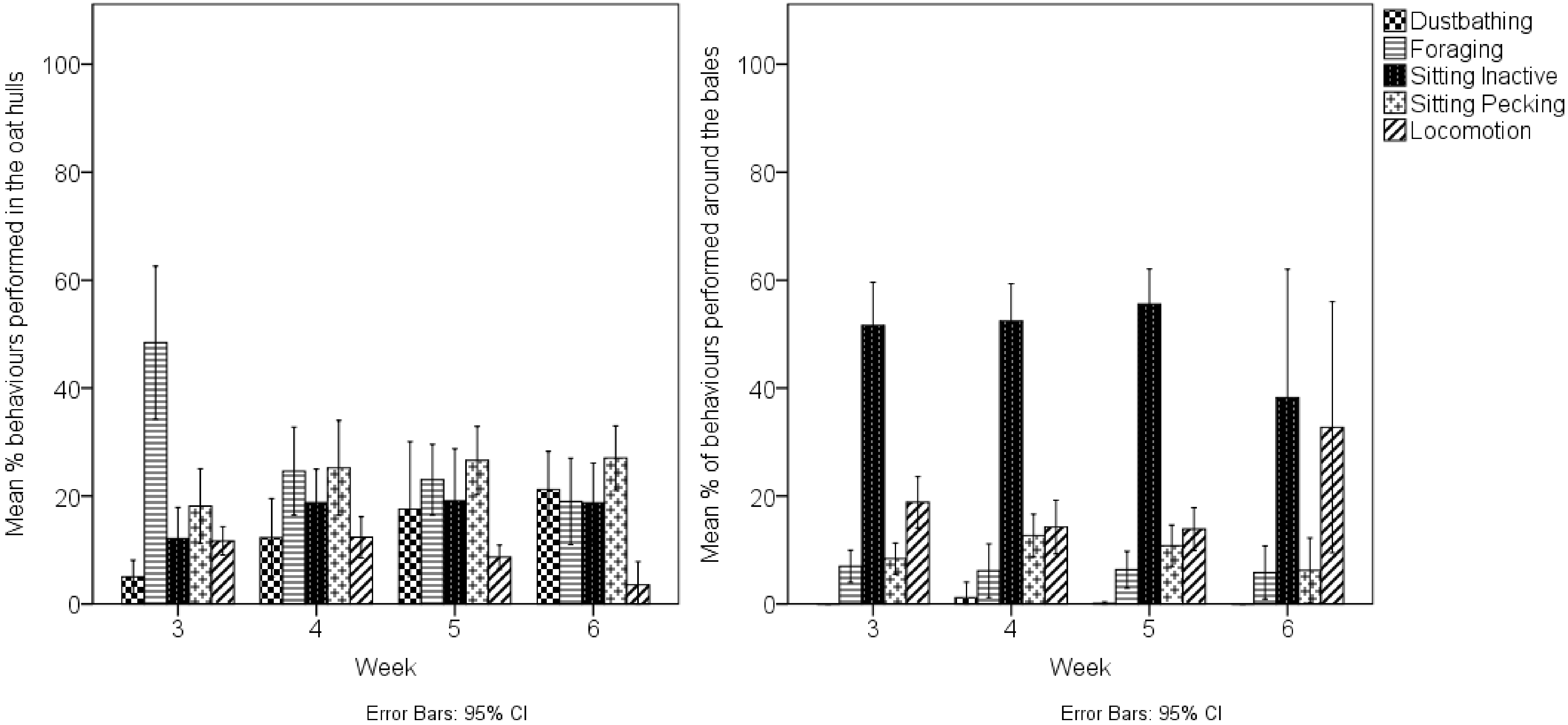

Figure 3. The effect of age on the mean % of behaviours performed in the oat hulls (left) and around the straw bales (right).

Table 10. Distribution of the frequencies of gait score (%)

Treatment	GS0	GS1	GS2	GS3	GS4	GS5
	Week 3					
Rings	69.4	26.3	4.4	0	0	0
Bales	69.4	28.8	1.9	0	0	0
Rings + Bales	66.3	33.1	0.6	0	0	0
Control	65.0	33.8	1.3	0	0	0
	Week 4					
	GS0	GS1	GS2	GS3	GS4	GS5
Rings	13.1	66.3	20.0	0.6	0	0
Bales	13.1	61.9	17.5	1.9	0	0
Rings + Bales	16.3	57.5	26.3	0	0	0
Control	8.8	69.4	20.0	1.9	0	0
	Week 5					
	GS0	GS1	GS2	GS3	GS4	GS5
Rings	2.5	52.5	41.3	1.9	2.5	0
Bales	1.3	52.5	41.3	5.0	0	0
Rings + Bales	3.1	59.4	33.1	4.4	0	0
Control	0.6	56.3	39.4	3.8	0	0
	Week 6					
	GS0	GS1	GS2	GS3	GS4	GS5
Rings	0	43.1	45.6	8.8	1.3	1.3
Bales	0	33.1	61.9	5.0	0	0
Rings + Bales	0	43.8	44.4	11.3	0.6	0
Control	0	26.9	62.5	10.0	0.6	0

[1]GS0 = gait score 0, GS1 = gait score 1 etc.

3.4 Discussion

In this trial, providing broilers with oat hulls, both in combination with straw bales and as a stand-alone dustbathing enrichment, led to an improvement in gait score in the final week of the production cycle. Birds in close proximity to the oat hulls and straw bales show a marked difference in the way they use the enrichments, with more foraging and dustbathing performed in oat hulls, and more sitting inactive observed around straw bales. When provided together, there was no effect on the level of use of adjacent oat hulls and straw bales compared to when they were provided in separate houses. A significant effect of treatment on the behaviour of broilers away from the enrichments was found, although our findings contradict previous research that showed an increase in activity (Kells et al., 2001). Conversely, this study found a decrease in locomotion and an increase in sitting behaviours in all enriched treatments compared to the unenriched control.

Broilers with access to oat hulls, in the OH and OH+B treatments, recorded better gait scores compared to those housed in the C treatment with no enrichment. Birds housed with only straw bales fell somewhere in the middle, with slightly lower gait scores than those in the control treatment (P = 0.79) and slightly higher scores to those recorded in the OH and OH+B treatments (P = 0.57). Broilers are particularly susceptible to skeletal disorders that impair mobility and can spend up to 76-86% of their time sitting down by slaughter weight (Weeks et al., 2000). This inactivity can, in turn, lead to a worsening of leg health and additional damage such as contact dermatitis (Bessei, 2006). These disorders are assumed to be painful and birds with gait scores >2 are considered to have poor welfare (Vestergaard and Sanotra, 1999; Danbury et al., 2000). When young broilers are forced to exercise they show a reduction in leg abnormalities by slaughter age, supporting the link between inactivity and poor leg health (Thorp and Duff, 1988; Bessei, 2006). Promoting activity in broilers has been attempted practically by increasing the distance between feeders and drinkers, which led to increased locomotion and improved leg condition (Reiter and Bessei, 1996). Increasing broilers environmental complexity with barriers, perches and straw bales has been shown to increase activity (Kells et al., 2001; Bizeray et al., 2002a). Providing oat hulls may have acted in a similar manner, by providing broilers with an opportunity to exercise and improving the incidence of poor leg health. Dustbathing is an active behaviour that begins with birds scratching at the ground before squatting on the substrate with their feathers erect. The birds then use leg kicks, scratches and vertical wing shakes (an upward shuffling motion) to move dust into their feathers (van Liere et al., 1991). The leg and body movements involved may have helped to develop bone and muscle conformation, leading to an improvement in leg health by slaughter weight (Sandusky and Heath, 1988; Rutten et al., 2002; Bessei, 2006).

Contrary to our expectations, there were higher levels of locomotion and less sitting inactive in the control treatment compared to unenriched areas of OH, B, OH+B treatments. Considering the improvement in gait score in enriched treatments, it seems unlikely that the reduction in activity was as a result of poorer leg health. It is possible that the presence of the straw bales and/or oat hulls in the enriched treatments led to a reduction in the amount of time birds spent exploring to find suitable resources for foraging and dustbathing (Nicol and Guildford, 1991). However, broilers time budgets are fairly inflexible in different enrichment conditions (Shields et al., 2005), and it may be that providing enrichments that promote exploratory and dustbathing behaviour creates areas of activity in enriched treatments, and that unenriched areas are primarily used for rest. It is therefore difficult to establish whether overall levels of activity by broiler chickens were affected by treatment in the current study. Previous trials have provided broilers with varying numbers of straw bales and reported either an increase in overall activity (Kells et al., 2001) or no effect on any behaviours (Bailie et al., 2013; Bailie and O'Connell, 2014). It is likely that the discrepancy in bale density can account for the difference in results. Kells et al. (2001) reported an increase in locomotion and standing, and a decrease in sitting and resting in houses with bales compared to barren housing. Their enriched houses contained a high density of straw bales (118 in one house and 81 in another; 1 bale per 17 m^2), compared to the current trial (9 bales at any one time and 46 across the cycle, equating to 2 / 1 000 birds; 1 per 155 m^2). There has been little research on different levels of bale provision, however Bailie and O'Connell (2014) found no improvement in bird welfare when broilers were housed with 1 bale per 29 m^2 (2 bales per 1 000 birds at all times) compared to a lower 1 bale per 44 m^2 (1.3 bales per 1 000 birds at all times). Currently protocols for enriched housing in the UK (usually 1.5-2 bales per 1 000 birds) were largely developed within the limitations of what could practically be implemented on farms at the time, however further research on the optimal level of bale provision would be useful.

The behaviour of broilers in close proximity to the oat hulls or straw bales was considerably different. Birds performed more dustbathing, foraging and sitting pecking in oat hulls compared to when they were around straw bales. Oat hulls are a loose, friable substrate and possess qualities similar to peat and sand, for which broilers show a preference for foraging and dustbathing (e.g. Petherick and Duncan, 1989; Shields et al., 2004). Although all straw bales provided during the trial were dismantled, which means birds did peck and scratch the straw throughout the cycle, levels of foraging behaviour observed around the bales were low. Foraging in broilers is a relatively short behaviour, with average foraging bouts lasting around 3 minutes (Bizeray et al., 2002b), which may have been missed by scan sampling. However, the high levels of sitting observed around bales suggest they may serve another positive function by providing cover and perceived protection for broilers. Similar clustering around long-cut straw bales has been reported (Kells et al., 2001; Bergmann et al., 2017), which suggests plastic wrapped straw bales can fulfil a similar role as cover. Increased levels of resting and preening are observed in hens provided with cover panels, probably because they would be particularly vulnerable to predation while their eyes are closed (Newberry and Shackleton, 1997; Cornetto and Estevez, 2001b). Homogeneity of distribution of broilers is also improved with the presence of cover, as birds have a tendency to group near pen walls (Cornetto and Estevez, 2001a). There was also a difference in the way the two enrichments were used over time. While foraging and dustbathing remained low around straw bales, in oat hulls there was a reduction in foraging over time and an increase in dustbathing between weeks 3 and 6. Consistent with previous research (e.g. Dawson and Siegel, 1967), there was also a reduction in foraging in the unenriched areas of the house. Foraging is also an example of contrafreeloading, whereby an animal with easy access to food will choose to work for food (Osbourne, 1977). Broilers have constant access to food and are less likely to perform contrafreeloading behaviours compared to laying hens and their Red Jungle Fowl ancestors (Lindqvist et al., 2006), and this may especially become the case when the activity requires more energy in older and heavier birds. Dustbathing has a different motivational basis and there was an increase in the level of dustbathing between weeks 3 and 6, which corresponds with some previous studies (Weeks et al., 1994; Bokkers and Koene, 2003). These results suggest that a dustbathing substrate may be a more suitable enrichment for birds to engage with for the entirety of the production cycle.

Straw bales and oat hulls may serve different functions within a commercial house and therefore may be compatible enrichments if provided together. There was no effect on the types of behaviours performed with oat hulls or bales when they were placed near to the alternative enrichment (in the OH+B treatment), compared to when they were placed in individual houses (OH or B treatments). Both enrichments still continued to attract the same number of birds in single and combined conditions, which suggests there would be no impact on straw bale use if oat hulls were provided as a supplementary enrichment. There were also no negative effects on production or any environmental parameters of combining both enrichment types. It is important that enrichments have no negative effect on productivity in order for them to be successfully introduced commercially. Previous nutrition studies have found ground oat hulls to have no negative effect on broiler weight gain and to actually improve feed consumption and conversion efficiency (Hetland and Svihus, 2001; Hetland et al., 2003). There was also no effect of treatment on ammonia levels or litter quality, and no influence on the percentage of pododermatitis recorded. The dry nature of the oat hulls was expected to improve litter quality, and therefore reduce incidences of pododermatitis (Bilgili et al., 2009), however its restriction to rings around the house is likely to have limited its effectiveness in this respect. Although dust levels were not monitored in this study, previous reports of problems with the dustiness of oat hulls have been reported (Meyer et al., 2007), and should be considered in further trials.

3.5 Conclusions

In conclusion, oat hulls were successful at promoting dustbathing in commercially housed broilers and maintained interest throughout the production cycle. Oat hulls were more successful in promoting foraging and dustbathing compared to straw bales, however bales were pecked at throughout the trial and were fully dismantled in each cycle. The high number of birds sitting around the bales suggests their additional value as protective cover for the birds. Importantly, broilers housed with oat hulls as a dustbathing enrichment, both singly and in combination with straw bales, had better gait scores than those in the control treatment with no enrichment.

This demonstrates an effect of including a dustbathing substrate on the leg health of broiler chickens. However, there was no increase in activity observed in unenriched areas when broilers were given enrichments, which offers conflicting results to previous research. Indeed, there was an increase in activity in the control treatment compared to unenriched areas of the enriched treatments, which may be due to birds using areas away from enrichments primarily for rest. There was no effect on the level of use of each enrichment, and no negative effect on performance or environmental measures, when both types of enrichment were provided together. This suggests oat hulls would be suitable as a supplementary enrichment to straw bales, and they appear to satisfy distinct motivations for broilers. Some practical issues have been outlined that must be considered for this substrate to be introduced commercially. Further research and collaboration with commercial suppliers will be useful to find the most effective way of incorporating a dustbathing enrichment into commercial housing.

Chapter Four

Study 3

Is there any benefit to grouping environmental enrichments in commercial broiler housing?

Preface

In “higher welfare” housing, broiler enrichments are usually placed evenly around the house in typically low densities. As little is known about the home ranges of commercially housed broilers, this spread of enrichments increases the likelihood that birds will encounter and benefit from these additions. However, some research has shown that laying hens are more likely to interact with a pecking device if it consisted of several different materials (Jones et al., 2000). Broilers were also attracted to a peripheral area of a pen when presented with a variety of enrichments simultaneously (a straw bale, peat, a platform and a ramp; Newberry, 1999). It was therefore decided to test whether broilers would be more attracted to grouped “enrichment areas” compared to single enrichments and whether this would increase the overall level of enrichment use.

There was some interest from the commercial producer in developing an appropriate pecking stimulus for broiler chickens, and previous commercial scale research had noted a higher interest in bunches of string (Bailie and O’Connell, 2015) than previously reported in smaller studies (Arnould et al., 2004). In addition, there is some evidence that poultry will perform more dustbathing and comfort behaviours in the presence of vertical cover panels (Cornetto and Estevez, 2001b). As demonstrated in previous chapters, straw bales appear to be an attractive area to rest for broilers, suggesting their value as protective cover. It was hypothesised that more dustbathing behaviour might be observed in dustbathing areas bordered by straw bales. Therefore, the enrichments chosen for this experiment included straw bales, rings of oat hulls, and plastic-coated chains (as a pecking enrichment). This study was conducted at a commercial level, in order to best determine the practicalities and effects of such a modification. This limited the number of replications possible and this study is intended as a pilot trial to guide future research.

Abstract

This experiment explored whether creating 'enriched areas' would attract more broilers and stimulate a higher level of use compared to providing individual enrichments. Approximately 56 000 Ross 308 broiler chickens were placed in two matched commercial houses (30 kg/m^2) on one farm. On day 4, three enrichment types were supplied: plastic-wrapped straw bales (SB), oat hulls (OH) provided in steel rings (1.1 diameter, 7.62 cm deep), and black/yellow plastic pecking chain (Pe) hanging from feeder lines. These enrichments were grouped into seven enrichment combinations per house: 1) SB only, 2) OH only, 3) Pe only, 4) SB+OH, 5) SB+Pe, 6) OH+Pe, and 7) SB+OH+Pe. The farm was visited twice weekly over one production cycle in weeks 2, 3, 4 and 5. Level of enrichment use was assessed using video footage taken of each enrichment area. Scan sampling was conducted of broilers within 0.4 m of the straw bales and within the borders of the steel oat hulls rings, with the percentage of broilers performing each behaviour calculated. Focal sampling was also used to record the number of pecks directed at the straw bales and pecking chain, and of the number of vertical wingshakes performed in the oat hulls. Each enrichment type was compared with its three alternative combinations, e.g. level of use of straw bales was compared between the SB, SB+OH, SB+Pe and SB+OH+Pe combinations. In general, level of use of SB and OH was similar to previous research, but use of Pe was higher than anticipated. Enrichment combination did not have a significant effect on the number of broilers around the straw bales or in the oat hull rings, or on the percentage of any behaviours observed. Focal observations of direct use of each enrichment revealed that significantly more vertical wingshakes were performed when the oat hulls were placed singly (OH) rather than in the SB+OH+Pe combination ($P = 0.026$). There was a significant interaction between enrichment combination and week for the number of pecks directed at the straw bales ($P = 0.013$), and no effect of enrichment combination on the number of pecks directed at the pecking chain ($P > 0.05$). Specific effects of placing SB close to OH (as a possible form of vertical cover) on levels of dustbathing and of disturbance to birds within these dustbathing rings was examined, but no significant effects were found. In conclusion, there appeared to be no obvious benefits to grouping these enrichments together rather than providing them singly, and some practical benefits to placing enrichments individually (such as more even distribution of fresh straw into the litter throughout the house). Straw bales did not

appear to offer significant protective cover around dustbathing areas, with no increase in comfort behaviours or reduction in disturbances observed. Broilers were substantially more interested in the pecking enrichment than has been previously reported, highlighting the need for more commercial scale research.

4.1 Introduction

The use of environmental enrichment has become a common method of providing intensively farmed animals with more complex and diverse environments. Introducing resources that provide stimulation and improve the biological functioning of animals (Newberry, 1995) has resulted in a reduction in abnormal behaviours (D'eath et al., 2014; Tahamtani et al., 2016) and improvements in production (El-Lethey et al., 2000) and well-being (Douglas et al., 2012). However, there remains little information available on the most effective ways to provide enrichment to commercial broiler chickens, and research is often performed in laboratory settings which cannot replicate the conditions and difficulties encountered at a commercial scale (Dawkins et al., 2003). Provision of straw bales has been found to increase the levels of activity in a house (Kells et al., 2001) and to improve leg health (Bailie et al., 2013), however the low numbers of bales commonly provided at a commercial scale may mitigate this effect. The use of pecking enrichments to promote normal pecking and exploratory behaviours has been successful in reducing abnormal behaviours in laying hens (Gvaryahu et al., 1994; McAdie et al., 2005), and may help to improve walking ability in broilers (Bailie and O'Connell, 2015). However, broilers can show a low level of interest in such enrichments (Arnould et al., 2004). Previous chapters have demonstrated that broilers do show a sustained interest in appropriate dustbathing materials. Broilers will readily perform dustbathing in a loose, friable substrate such as peat or oat hulls, and show increased use of dustbathing substrates across the production cycle.

Animals will show greater interaction with their environment when their surroundings are complex rather than simple (Chamove, 1989), and broilers are motivated to explore areas with novel items and non-essential resources (Newberry, 1999). In enriched housing, chickens are usually offered enrichments separately, however laying hens will peck more readily at three pecking stimuli when offered simultaneously rather than individually (Jones et al., 2000). It was therefore hypothesised that grouping enrichments together would attract a higher number of birds to the area and result in a higher level of enrichment use compared to single enrichments. This trial was conducted in commercial housing in order to better understand the practicality of this method; straw bales, plastic coated pecking chains, and rings filled with oat hulls as a dustbathing substrate were offered to the birds in various combinations, with the level of use and types of behaviours performed around each enrichment observed throughout the production cycle.

In addition, previous trials have found that birds will group and rest around straw bales (Kells et al., 2001; Study 2) indicating their possible value as protective cover. Adaptive anti-predator behaviours have persisted in domesticated fowl and birds can show strong fear reactions to sudden events and run for cover in the absence of genuine predators (Evans et al., 1993a Evans et al., 1993b; Dawkins et al., 2003). Both laying hens and broiler chickens show a preference for areas enriched with some type of vertical cover that may offer perceived protection (Newberry and Shackleton, 1997; Cornetto and Estevez, 2001a; Dawkins et al., 2003). In large group sizes, broilers will perform more dustbathing, resting and preening in the presence of vertical cover (Cornetto and Estevez, 2001b), and when grouped next to a vertical structure birds are less likely to be jostled and disturbed by conspecifics (Cornetto et al., 2002), which could otherwise approach from all directions (Buijs et al., 2010). As such, our second hypothesis was that dustbathing areas bordered by straw bales would be more protected, and therefore an increase in dustbathing and comfort behaviours, and a reduction in disturbances would be observed in these grouped enrichment areas.

4.2 Materials and Methods

4.2.1 Subjects and housing

This trial was conducted in two matched houses on a Northern Ireland commercial farm between May and July 2017. Approximately 28 000 Ross 308 broiler chickens were placed “as hatched” in each house on the same day, giving an average 50:50 female to male ratio. The houses were matched for design and size; both were 85 m x 20 m metal framed sheds, with an average usable floor space of ~1 716 m^2. Birds were housed at a maximum stocking density of 30 kg/m^2, which was the standard for this farm. The houses were initially bedded on fresh woodshavings and additional woodshavings were distributed throughout to maintain litter condition at the farmer's discretion. Temperature, humidity and light levels were controlled automatically in the same manner as described in Chapter 2 (2.2.1)

4.2.2 Treatments and experimental design

Seven enrichment combinations were placed in each house (Figure 4): 1) individual straw bales only (SB), 2) individual oat hulls rings only (OH), 3) pecking chains only (Pe), 4) straw bales and pecking chains (SB+Pe), 5) oat hulls and pecking chains (OH+Pe), 6) straw bales and oat hulls (SB+OH), 7) straw bales and oat hulls and pecking chains (SB+OH+Pe; Photo 3). All enrichments were placed on day 4 of the cycle, which was earlier than previously described enrichment placement in this thesis, and was possible because of the farmers discretion that chick feeder sheets would be sufficiently cleared by day 4. Enrichment location was chosen using restricted randomisation, with the condition that enrichment areas should be evenly placed in back and front areas of the houses. All enrichments were equidistant from the nearest windows to control for the influence of natural light intensity. Straw bales were plastic wrapped, short cut straw bales that were used as standard enrichment bales on the farm. Three bales were placed in an L-shape which created a semi-enclosed area (Photo 3). As per normal management practices, the two long sides of the bales were cut open to allow birds to peck out the straw. For the purposes of this trial, bales were replaced in the same location once the top of the bale collapsed.

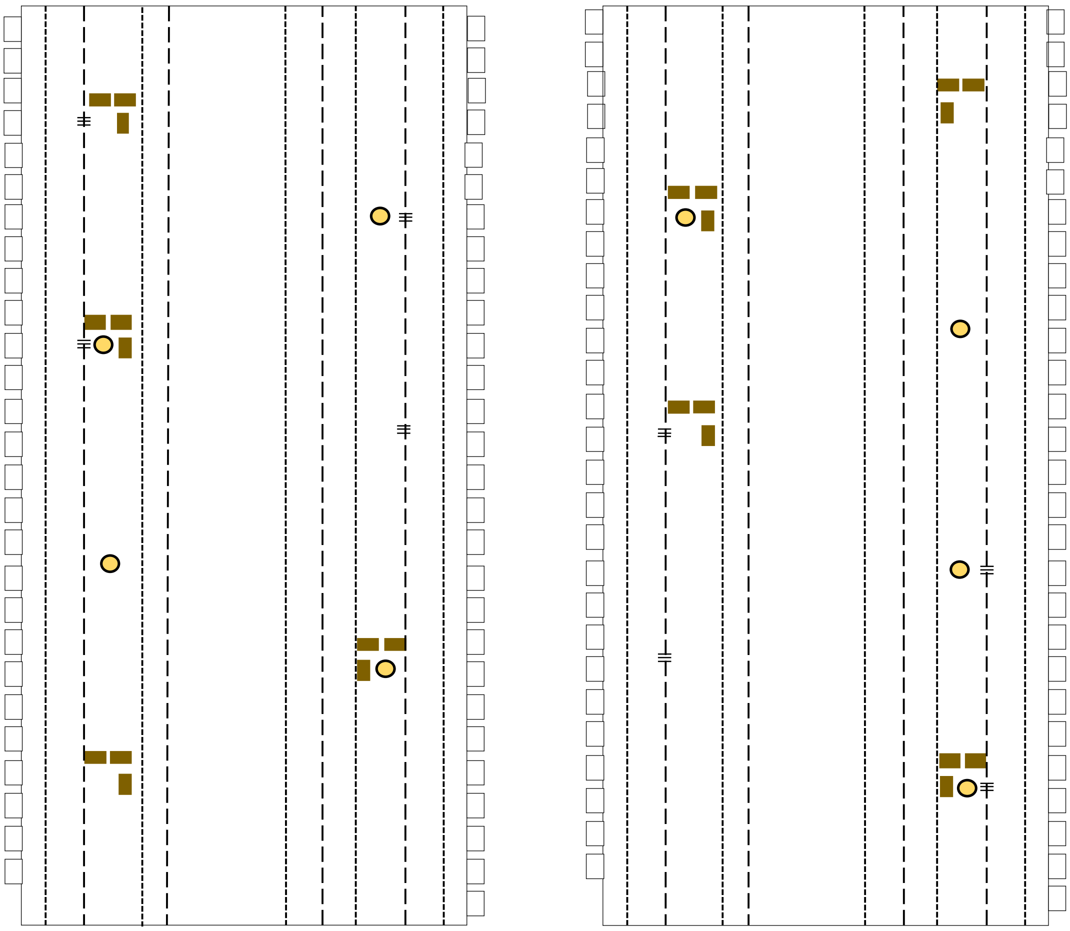

Figure 4. Placement of enrichment areas around matched houses. Vertical dotted lines represent nipple drinker lines; vertical dashed lines represent feeder lines. Boxes on the outer sides of the houses represent windows. The enrichments included were:

Straw bales   Rings of oat hulls   Pecking chain ☰

These enrichments were grouped into seven different enriched areas: 1) straw bales only, 2) oat hulls only, 3) pecking chain only, 4) straw bales and pecking chain, 5) oat hulls and pecking chain, 6) straw bales and oat hulls, 7) straw bales, oat hulls and pecking chain.

Oat hulls were locally sourced (Whites Speedicook Ltd, Craigavon, UK) and delivered in 1 tonne bags as previously described. Oat hulls were provided in one steel ring per area; steel rings had a 1.1 m diameter and were 7.62 cm deep, with an area of 0.95 cm^2. Birds were able to climb into the rings from day 4 and were unable to perch on the edges. Approximately 14 kg of oat hulls were initially placed in the rings, filling them to a depth of about 5 cm. All rings were topped up twice a week on the morning of observations throughout the trial to their original level. During weeks 4 and 5, oat hulls were also refilled on an additional day between observations to maintain their condition as the oat hulls degraded more rapidly in later weeks. The pecking chain provided was 8 mm black and yellow plastic-coated barrier chain, cut to lengths of approximately 30 cm (AIMTools Ltd, UK). Yellow has previously been found to be an attractive pecking colour to chickens (Jones and Carmichael, 1998; Jones et al, 2000). The chain was hung from the feeder lines, in three sections with two hanging chains per section, opposite the single bale in the enrichment area (Photo 3). The chains hung approximately 0.4 m from the edge of the oat hull rings, if present, and varied in distance from each straw bale (Photo 3). In areas with both straw bales and oat hulls, oat hull rings were approximately 0.5 m from the edge of the bales.

Photo 3. Photograph of enrichments placed in the SB+OH+Pe area (straw bales, oat hulls and pecking chain). Each individual enrichment placement represents the way enrichments are arranged in each combination, e.g. straw bales are arranged in an L-shape whether placed singly or in combination with other enrichments

4.2.3 Data collection

Both houses were visited twice a week during weeks 2, 3, 4 and 5 of the production cycle. Video footage of bird behaviour around each enrichment was taken between 09:00 h and 13:00 h, and on-farm measures of disturbance and light intensity were completed between 13:00 h and 16:00 h each day.

Camileo X-Sports cameras, mounted on 1 metre high wooden tripods, were used to record enrichment areas. Both houses were filmed on the same day, one after the other and the starting house was randomised for each observation. The footage was then analysed using a combination of scan sampling and focal observations. For straw bales, the two adjacent bales in all four locations were filmed on both sides simultaneously for 35 minutes, using eight cameras. Following a 5 minute settling period, birds directly in front of the bales (up to 0.4 m distance from the bales, measured as the height of the bale virtually transposed onto the house floor in front of the adjacent bales) were scan sampled at 10, 20 and 30 minutes of each video (a total of 384 scans). All birds in the area around the bales were recorded as either sitting inactive, sitting pecking, foraging, dustbathing, locomotion (walking or standing), preening or play (frolicking or sparring). The three scan samples were averaged to give the mean number of birds performing each behaviour in proximity to the two bales. Scores for the two sides of the bales were summed to give a total score, and the average number of birds performing each behaviour was then expressed as a percentage of the total number of birds around the bales. The bi-weekly observations were then averaged to give one value per week. Once per week, footage of each of the two bales was also observed for a 10 minute focal period (following a 10 minute settling period). The number of pecks directed at exposed straw on the side of the bales facing the inner area of the L-shape (facing towards other enrichments, if present) was recorded and values for the two bales summed, to give a score of the number of pecks directed at the inner side of two bales.

Oat hulls were similarly filmed for 35 minutes in each location, using a camera on a tripod set up next to each of the four rings. Each video was analysed using scan sampling to assess behaviour in the oat hulls, and focal sampling to measure the amount of dustbathing. For each video, scan sampling was performed in the same manner as around bales, with scans of bird behaviour taken at 10, 20 and 30 minutes after a 5 minute settling period. The three scans were then averaged to give the mean number of birds performing each behaviour, and the bi-weekly scores averaged to give one score per week. For weekly focal observations, following a 10 minute settling period, the number of vertical wing shakes performed in the oat hulls was counted during a 20 minute focal period as a measure of the amount of dustbathing performed (e.g. Sanotra et al, 1995). The mean length of a dustbathing bout in oat hulls is 14 minutes (SEM 0.85), with an average of 23 vertical wingshakes (Chapter 2). This length of focal observation was chosen in order to observe a number of complete dustbathing bouts.

Pecking chain areas were filmed for a total of 25 minutes each, using a camera on a tripod placed next to each of the four chain areas (facing towards the house wall). Due to the rapid nature of any engagement with pecking chains, only focal sampling was performed on pecking chain footage. Once a week, after a 5 minute settling period, the number of pecks directed at the six pecking chains per area was counted for a 10 minute focal period.

To assess whether bales act as cover for birds using oat hulls, a separate measure of disturbance was recorded directly by the same observer each week. The number of disturbance events was counted in birds in stand-alone oat hull rings (OH) and in oat hull rings surrounded by bales (SB+OH). The observer sat approximately 2 metres from the ring in both cases and, after a 5 minute settling period, recorded any incidences of disturbance for the following 10 minutes. An incidence of disturbance was recorded when a bird made physical contact with another bird, causing it to stand (Estevez, 1994; Cornetto et al., 2002). During the focal period, the number of birds in the ring was counted every minute, and the 10 scores were averaged to give the mean number of birds present in the ring during the observation period. This additional measure of ring occupancy was taken to allow direct comparison with disturbance events, in order to better understand the relationship between bird density in the rings and disturbance. The number of disturbance events recorded and the number of birds recorded in the ring were averaged to give one score per week.

Light levels were automatically maintained, however measures of light (lux meter) were taken from all enrichment areas in both houses, no more than 5 minutes apart, to monitor variation and avoid light as a confounding variable.

4.3.4 Statistical analysis

Data were analysed using SPSS (version 23). For each enrichment type, the four levels of enrichment combination were compared with each other; for example, data for oat hulls were compared between 1) individual oat hulls, 2) oat hulls and pecking chain, 3) oat hulls and straw bales and 4) oat hulls, straw bales and pecking chain. Normality of residuals was assessed for each data set through inspection of normality plots and Shapiro-Wilk tests. Significance level was set at $P < 0.05$.

For observations of % behaviours performed around the bales and in the oat hulls, the main and interaction effects of treatment and week were analysed using GLMM, with enrichment combination + week as fixed effects, and house.week as a random factor. Data from focal samples on the number of VWS performed in oat hulls ($n = 32$), the number of pecks directed at bales ($n = 32$), and the number of pecks directed at pecking chain ($n=32$) were analysed using the same model. Light intensity ($n=112$) was compared between the seven enrichment areas using GLM with enrichment combination as a fixed factor and house as a random factor, and between the two houses using a one-way ANOVA. There was no significant difference in ring occupancy between OH and SB+OH during focal observations of disturbance ($P > 0.05$), therefore disturbance was analysed using a GLM with enrichment combination as a fixed factor and house as a random factor ($n = 16$). A Pearson product-moment correlation coefficient was calculated to investigate the linear relationship of average birds in the ring and the number of disturbances recorded.

4.3 Results

There was no significant difference in light intensity between enrichment combinations or between houses ($P > 0.05$). Light intensity was therefore disregarded as a source of variation. Play behaviours were very infrequently recorded and were excluded from analysis.

4.3.1 Straw Bales

A total of 5370 broilers were observed and categorised around the bales during the trial. Whether bales were placed singly or in combination with other enrichments did not have a significant effect on the total number of birds counted around the bales. Overall, there was an average of 30 (SE = 1.1) birds counted around the two bales, with fewer birds counted around the bales as birds aged ($F_{3,3} = 17.46$, $P = 0.009$), which was expected because the observation area was fixed so fewer birds fit within the scan area. Although there was minimal dustbathing performed around the straw bales (M = 0.3%, SE = 0.10), there was a significant interaction between enrichment combination and week ($F_{9,12} = 2.92$, P = 0.043). Simple effects analysis showed that the broilers age had a significant effect on the amount of dustbathing performed in the SB+OH (week 2, M = 0%; week 3, M = 0%; week 4, M = 1.1%; week 5, M = 1.1%) and SB+OH+Pe (week 2, M = 0%, week 3, M = 0.3%, week 4, M = 1.5%, week 5, M = 0%) but not the SB (week 2, M = 0%; week 3, M = 0.26%; week 4, M = 0%; week 5, M = 0% and SB+Pe combinations (week 2, M = 0%; week 3, M = 0.81%; week 4, M = 0%; week 5, M = 0.34%). There was no significant effect of enrichment combination on percentage of broilers sitting inactive, sitting pecking, foraging, preening or in locomotion around the bales (Table 11), and no significant interactions between combination and week. There was, however, a main effect of age on the % of sitting pecking around the bales ($F_{3,4} = 6.66$, P = 0.049), with significantly more sitting pecking observed in week 2 compared to week 5 (week 2, M = 14.1%, SE = 1.02; week 3, M = 11.8%, SE = 5.13; week 4, M = 8.1%, SE = 1.34; week 5, M = 5.6%, SE = 0.69).

During the 10 minute focal period, there were an average of 310 (SE = 18.8) pecks directed at the straw bales (Figure 5). There was a significant interaction between enrichment combination and week for the average number of bale pecks ($F_{9,12}$ = 4.08, P = 0.013). Average bale pecking was significantly affected by enrichment combination in weeks 2 and 4, with higher levels of pecking at the SB compared to the SB+OH+Pe combination in week 2 (SB, M = 451.0, SE = 18.0; SB+OH, M = 278.0, SE = 50; SB+Pe, M = 395.5, SE = 3.65; SB+OH+Pe, M = 207.0, SE = 0.98), and higher levels of pecking in the SB+Pe combination compared to SB+OH in week 4 (SB, M = 309.5, SE = 122.5; SB+OH, M = 133.5, SE = 43.5; SB+Pe, M = 375.0, SE = 47.0 ; SB+OH+Pe, M = 325.0, SE = 118.0).

4.3.2 Oat Hulls

A total of 2161 broilers were observed and categorised in the oat hulls rings. Overall, an average of 11 birds were counted in the rings; the birds age did not affect how many birds were present in the rings ($P > 0.05$). Whether the oat hulls were placed singly or in combination with other enrichments also did not have a significant effect on the total number of birds in the rings or the percentage of any behaviours performed ($P > 0.05$). There was a significant effect of broiler age on the percentage of birds dustbathing ($F_{3,4} = 14.44$, P = 0.013), with levels of dustbathing increasing as birds aged (week 2, M = 9.2%, SE = 1.56; week 3, M = 17.7%, SE = 6.8; week 4, M = 27.2%, SE = 2.20; week 5, M = 31.9%, SE = 4.29). There was also an overall effect of age on the percentage of broilers sitting inactive in the rings, with the most inactivity recorded in week 5 ($F_{3,4} = 10.66$, P = 0.022; week 2, M = 27.6%, SE = 2.01; week 3, M = 31.0%, SE = 3.19; week 4, M = 21.7%, SE = 2.81; week 5, M = 31.4%, SE = 1.31%).

There was an average of 74 (SE = 8.43) vertical wingshakes per 20 minute focal period. Enrichment combination had a significant effect on the number of vertical wingshakes performed ($F_{3,12} = 4.44$, P = 0.026), with significantly more vertical wingshakes performed in the OH only compared to SB+OH+Pe areas (OH, M = 93.6, SE = 19.86; OH+Pe, M = 71.1, SE = 14.96; SB+OH, M = 87.6, SE = 11.82; SB+OH+Pe, M = 41.5, SE = 15.81; Figure 5). There was a significant main effect of week on the percentage of birds dustbathing in the rings ($F_{3,4} = 9.12$, P = 0.029). Pairwise comparisons indicated a significant increase in vertical wingshakes between weeks 2 and 3, but no significant differences between weeks 2, 4 and 5 or weeks 3, 4 and 5 (week 2, M = 41.5, SE = 9.86; week 3, M = 106.9, SE = 21.11; week 4, M = 85.1, SE = 13.55; week 5, M = 63.0, SE = 13.79).

There was a positive correlation between the average number of birds present in the ring and the number of disturbance events recorded ($r(14) = 0.88$, $P < 0.001$) and, in agreement with data from scan samples of behaviour, no significant difference in the number of birds counted in the OH compared to the SB+OH during focal observations. There was no significant effect of the presence of straw bales on the level of disturbance in the oat hulls ($P > 0.05$; SB+OH, M = 2.4, SE = 0.65; OH, M = 4.5, SE = 1.09).

Table 11. The behaviour of broilers using enrichments placed in each combination (mean % ± standard error)

	Enrichment combination				Treatment	Age	Treatment*Age
	OH	SB+OH	OH+Pe	SB+OH+Pe	*P* value	*P* value	*P* value
Behaviour in oat hulls (%):							
Sitting inactive	26.58 ± 3.36	29.03 ± 1.69	28.07 ± 3.22	28.03 ± 2.74	ns	0.022	ns
Sitting pecking	15.66 ± 1.93	17.35 ± 2.38	23.52 ± 4.71	19.39 ± 2.27	0.071†	ns	0.096†
Foraging	17.06 ± 1.94	14.71 ± 2.23	14.40 ± 1.31	15.31 ± 2.42	ns	ns	ns
Dustbathing	24.40 ± 5.41	24.91 ± 4.11	19.19 ± 3.57	17.53 ± 3.25	ns	0.013	ns
Locomotion	9.70 ± 2.15	10.89 ± 1.81	10.67 ± 0.86	14.20 ± 3.34	ns	ns	ns
Preening	6.60 ± 0.76	3.12 ± 0.93	3.91 ± 0.46	5.54 ± 0.96	ns	ns	ns
	SB	SB+OH	SB+Pe	SB+OH+Pe			
Behaviour around straw bales (%):							
Sitting inactive	49.32 ± 2.89	51.80 ± 3.53	49.41 ± 3.68	48.44 ± 3.00	ns	ns	ns
Sitting pecking	9.91 ± 1.80	9.62 ± 1.86	9.14 ± 1.40	11.02 ± 1.96	ns	0.049	ns
Foraging	8.58 ± 1.00	8.67 ± 1.12	9.63 ± 1.06	8.63 ± 0.63	ns	ns	ns
Dustbathing	0.064 ± 0.064	0.56 ± 0.24	0.29 ± 0.21	0.45 ± 0.25	ns	ns	0.043
Locomotion	25.14 ± 1.89	23.81 ± 2.10	26.53 ± 3.52	26.83 ± 2.49	ns	0.086†	ns
Preening	6.29 ± 0.91	4.84 ± 0.97	4.87 ± 0.88	4.64 ± 0.55	ns	ns	ns

† $P < 0.1$

Oat hulls rings placed singly (OH) with straw bales (SB+OH), with pecking chain (OH+Pe), or with straw bales, oat hulls and pecking chain (SB+OH+Pe); straw bales placed singly (SB), with oat hulls (SB+OH), with pecking chain (SB+Pe), or with oat hulls and pecking chain (SB+OH+Pe).

4.3.3 Pecking Chain

A total of 2248 pecks were directed at the pecking chain during observations, with an average of 70 (SE = 3.9) pecks during each 10 minute focal period. The presence of other enrichments did not significantly affect the amount of chain pecking recorded (*P* > 0.05; Figure 5).

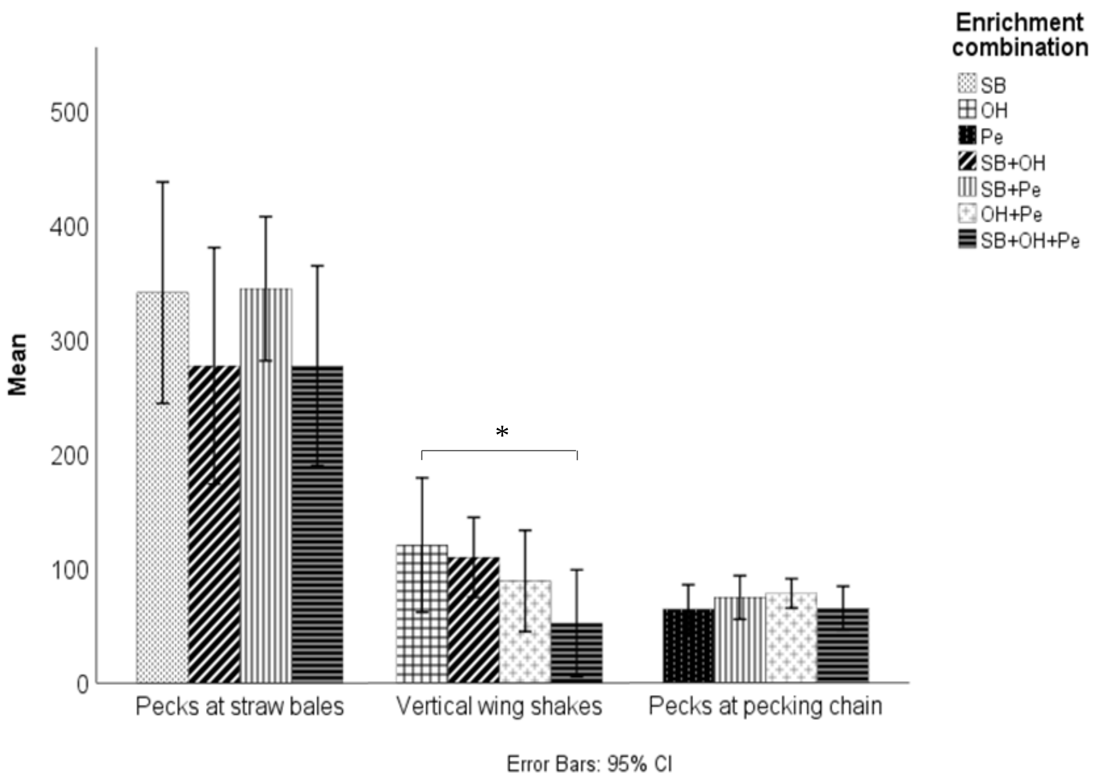

Figure 5. Focal observations of enrichment use. The average pecks directed at the straw bales when placed singly (SB), with oat hulls (SB+OH), with pecking chain (SB+Pe), or with oat hulls and pecking chain (SB+OH+Pe). The number of vertical wingshakes performed in oat hulls when placed singly (OH), with straw bales (SB+OH), with pecking chain (OH+Pe) or with both enrichments (SB+OH+Pe). The number of pecks directed at pecking chain when placed singly (Pe) and in various combinations with straw bales and oat hulls. *denotes a significant difference (P < 0.05).

4.4 Discussion

The aim of this experiment was to determine whether grouping enrichments together would affect the way in which they were used, in order to look for any obvious benefits of creating “enrichment areas” over placing separate enrichments around the house. Contrary to expectations, the more complex enrichment areas did not appear to be more attractive to broilers. Whether enrichments were placed singly or in combinations did not have an effect on the number of broilers in the oat hulls or around the straw bales, or on the overall percentage of each behaviour observed. There was, however, significantly more vertical wingshaking recorded in the single dust baths compared to those placed with several other enrichments. As broilers aged there was a predictable effect on several behaviours, with an increase in inactivity and dustbathing in the oat hulls, and a reduction in pecking around the bales (Chapter 2; Chapter 3). There was also a positive correlation between the density of broilers using the oat hulls and the average number of birds being disturbed, which is consistent with previous trials (Hall, 2001; Buijs et al., 2010).

Laying hens peck more readily at three types of pecking stimuli (string, beads and chain) when presented simultaneously compared to singly (Jones et al., 2000), which the authors suggest is because the varied stimuli are more effective at attracting the birds Our results suggest that grouping straw bales, oat hulls and pecking chain together did not increase the attractiveness of these enrichments. There was no increase in the number of broilers in the dustbathing areas or around the straw bales when they were placed in combination with other stimuli. There are several reasons why these enrichment areas may have failed to attract a higher number of birds than separately placed enrichments, although it is difficult to draw clear conclusions in this study. Unlike in Jones et al. (2000), where three types of the same pecking stimulus were presented, in the present trial the three different enrichments provided for different motivations. Oat hulls were attractive as a dustbathing substrate, while straw bales were largely used as a resting area, and the plastic-coated chain acted as an interactive pecking enrichment. The broilers may have been stimulated to use each enrichment regardless of nearby resources. It is also possible that broilers are less likely to engage with several enrichments when offered due to physical (Bessei, 2006) and motivational (Lindqvist et al., 2006) limitations. For example, an average foraging bout for broilers lasts only 3 minutes (Bizeray et al., 2002c), which may only be directed at one type of enrichment before a period of rest. It is also possible that the enrichments were too far apart to act as a clustered set of diverse stimuli.

Overall, the types of behaviours observed around the straw bales and inside the rings were largely unaffected by grouping enrichments together. There was an effect of enrichment combination on the amount of dustbathing performed around the straw bales, with the presence of nearby oat hull rings influencing levels of dustbathing performed around the adjacent straw bales as birds aged. This effect was probably due to some oat hulls being kicked into the space in front of the bales or by visual contact with dustbathing birds in the nearby rings (Petherick et al., 1995). There were no differences in the amount of sitting, foraging, locomotion or preening when either straw bales or oat hulls were placed with other enrichments. Focal observations of the number of pecks directed at the straw bales and pecking chain, and the number of vertical wingshakes performed in the oat hulls were used to assess direct enrichment use. There was a significant interaction between enrichment combination and broiler age for the number of pecks directed at straw bales, however this effect was inconsistent and varied over time. Enrichment combination did not influence the number of pecks directed at the pecking chain throughout the cycle. For oat hull use, despite enrichment combination not influencing the percentage of broilers engaged in dustbathing bouts, focal observations revealed that significantly more vertical wingshakes were performed when oat hulls were placed individually (OH, $M = 93.6$) rather than with both other types of enrichment (SB+OH+Pe, $M = 41.5$). The amount of vertical wingshaking observed has previously been used to identify substrate preferences for dustbathing (e.g. Sanotra et al., 1995) and our results may indicate that broilers may prefer to dustbathe in oat hulls placed without nearby straw bales or pecking chain. This was contrary to our hypothesis that straw bales bordering the dustbathing areas would provide protective cover, which would lead to an increase in dustbathing and reduction in disturbances. Provision of vertical cover has been shown to increase the levels of dustbathing, resting and preening in broilers (Cornetto and Estevez, 2001a; Newberry and Shackleton, 1997), probably because birds seek cover to perform behaviours that obscure their vision. Artificial cover has also been found to draw birds away from interior walls and reduce the overall levels of disturbance in a group (Cornetto et al., 2002). However, our observations showed no significant difference in the number of broilers jostled when dustbathing areas were bordered by straw bales. The straw bales used in the present trial (0.4 m high, 0.4 m wide) were not substantially smaller than cover panels (0.61 m high, 0.61 m wide) used in previous experiments (Cornetto and Estevez, 2001a). However, they may not have provided the extending vertical cover that birds show a preference for, for example the protection offered by trees and bushes (Dawkins et al. 2003). Straw bales may also have provided "obstructive" cover, which is less attractive than structures that provide partial concealment, possibly because opaque cover could conceal nearby predators (Newberry and Shackleton, 1997).

As broilers aged, levels of dustbathing and inactivity increased in the oat hulls and levels of sitting pecking increased around the straw bales, which is consistent with previous studies (Baxter et al., 2017; Baxter et al., 2018). The overall level of use of straw bales and oat hulls is comparable to previous research, with slightly less foraging in oat hulls in the present study (15-17% of birds observed were foraging) compared to previous reports (27-29%; Baxter et al., 2017; Baxter et al., 2018). The number of birds using the oat hulls for dustbathing appears to be fairly consistent, with 18-24% of birds observed dustbathing in the present trial and 13-19% in previous studies (Baxter et al., 2017; Baxter et al., 2018). Of the limited amount of studies that have looked at straw bale use in commercially housed broilers, their differences in methodology, housing and bale type make it difficult to draw direct comparisons. Kells et al. (2001) and Bailie et al. (2013) used long-cut straw bales and plastic wrapped straw bales respectively, both counting the number of birds on one side of the bale and doubling it for analysis. Kells et al. (2001) counted any birds clustering around the bale within a 180o angle, not specifying distance, and observed an average of 54 birds clustering around the bales in week 2, 22 in week 3 and 28 in week 4. Bailie et al. (2013) recorded birds 1 m distance from one side of the bale, reporting an average of 73 birds around the bales. They also found that birds were more likely to cluster around bales when provided with natural light in windowed housing which Kells et al. (2001) presumably did not use (not mentioned) and may explain the slightly lower numbers. In the present study, both sides of two adjacent bales were observed simultaneously, giving a more definite representation of the total number of birds around the bale. We observed an average of 30 birds within 0.5 m of the bales at any one time. It appears that birds are fairly consistent in their clustering around straw bales, suggesting they attract birds successfully in different systems and on different farms.

There was relatively high interest in the plastic chain, with an average of 70 pecks directed at the chain during the 10 minute observation period. White and yellow string has previously been identified as an attractive pecking stimulus for laying hens (Jones and Carmichael, 1998; Jones et al., 2000). However, when white string was offered to broilers housed in pens (2 m by 6 m) in groups of 40 (with access to woodshavings and sand), only 42 pecks were directed to the string during a total of 28 hours of observation (Arnould et al., 2004). Bailie and O'Connell (2015) reported that broilers housed commercially had more interest in the string than previously thought, with a bout of pecking directed at white string occurring every 78 seconds. In agreement with a recent review of broiler enrichment research (Riber et al., 2017), it appears that more on-farm research of broiler enrichments is needed to confirm the results of smaller trials. The plastic chain supplied in this trial may also be a more practical enrichment for commercial broiler housing than, for example, string (Jones et al., 2000), because it can be washed and re-used between production cycles.

There seemed to be no negative effects of presenting broilers with combinations of enrichments. In caged mice, presenting enrichments in a cluster rather than singly increased the amount of aggression, displacement of one animal using the enrichment by another, and stereotypic behaviours (Akre et al., 2011). Very little aggression was observed throughout the trial, which is consistent with previous findings (Mench, 1988; Pettit-Riley et al., 2002), and there was no increase in the amount of birds disturbed in combined enrichment areas. However, as commercial broiler houses contain a large number of animals, it is likely to be of more benefit to spread enrichments around the housing to impact more birds. It was also noted that placing bales in one area consistently throughout the trial led to an accumulation of dry straw in those specific areas, limiting the spread of fresh straw around the house. As enriched commercial farms can only supply a limited number of straw bales, distributing them evenly or in areas of wet litter may be more effective at maintaining litter condition.

4.5 Conclusions

In conclusion, there appeared to be no obvious benefits to clustering enrichments compared to offering them singly to birds. Grouping enrichments together did not attract a higher number of birds to use the enrichments or clearly affect the majority of behaviours performed in enriched areas. Indeed, more vertical wingshaking was observed in singly placed dustbathing areas and we found some practical advantages to spreading enrichments evenly throughout the houses. Although birds grouped and rested around the straw bales, there were no significant “protective” effects of increased dustbathing or preening when bales were present around oat hulls, and no reduction in disturbances. Further large-scale research applying different enrichment combinations in different houses would be useful to look for overall effects on behaviour and productivity. Broilers did show significantly more interest in a pecking enrichment than has been reported previously, highlighting the need for more commercial scale research.

Chapter Five

Study 4

The effect of environmental enrichment on broiler play behaviours and fear responses in commercial housing

Preface

The studies described in previous chapters have broadly been designed to investigate the effectiveness of enrichments in terms of activity levels, expression of natural behaviours, and leg health. However, little is known about whether environmental enrichments have a positive effect on broiler mental well-being. There has been a recent shift in thinking towards how we measure animal welfare, with the Five Freedoms being criticised for focusing on removing suffering rather than providing animals with a "life worth living" (FAWC, 2009; Wathes, 2010). It has, however, proved difficult to identify positive welfare indicators in poultry. The recent European Welfare Quality project developed recommendations of practical animal-based measures that could be used to assess broiler welfare (Welfare Quality, 2009). In this report, the authors highlight several key welfare questions to consider for any animal, including "does the behaviour of the animals reflect optimised emotional states?". They suggest several animal-based measures that correspond to this question, 1) expression of social behaviour (for which there has been no appropriate measure developed for broilers), 2) expression of other behaviours (a measure of range use only applicable to free range broilers), 3) good human animal relationship (measured using an avoidance test to rate fearfulness), and 4) positive emotional state. Positive emotional state is measured in this project using Qualitative Behaviour Assessment (QBA), in which an observer applies descriptors to animals based on their body language, these descriptors include 'comfortable', 'friendly', 'helpless' and 'scared'. This method has gained traction in recent years and makes use of humans intuitive understanding of animal postures and behavioural expression. However, identifying a behaviour that is associated with a positive emotional state, in the same way that fearfulness is associated with a negative emotional state, would be an important advance in monitoring broiler welfare. Play has been identified in a broad range of species as a behaviour associated with positive welfare (Ficken, 1977; Špinka et al., 2001; Burghardt, 2005). As discussed in Chapter 1, while scientists studying broilers have been reluctant to classify certain behaviours as play, there is some evidence that frolicking, sparring and food-running have qualities that resemble play seen in other species. In addition, increasing the complexity of an animal's environment using enrichment has been successful in inducing a positive emotional state in several species (Brydges et al., 2011; Douglas et al., 2012; Carreras et al., 2016). Therefore, this chapter explores a novel method of stimulating play behaviours, and compare the frequency of these behaviours between barren housing and enriched environments. In order to try to avoid providing broilers with modifications rather than enrichments (Newberry, 1995), features that broilers have previously shown a preference for were chosen, rather than standard commercial enrichments.

Abstract

Although providing environmental enrichment can improve broiler health and activity levels, there is limited understanding of the effect of these modifications on broiler experience. The main aim of this study was to investigate the emotional effects of providing broilers with environmental enrichment in commercial housing, by assessing levels of fearfulness and the frequency of behaviours that resemble play. There was also an interest in knowing whether the enrichments provided in this trial, platform perches and dust baths of peat, would have a positive effect on broiler activity levels. Broilers were assigned to one of three treatment houses over three production cycles: 1) platform perches, 2) platform perches + dust baths, and 3) barren control with no enrichment. Each house contained approximately 22 500 broilers. Six suspended platform perches (230 x 90 cm) were provided in Treatments 1 and 2, and four peat-filled dust baths (230 x 90 cm) in Treatment 2. Play behaviours and activity in unenriched areas of the house were measured in weeks 3, 4 and 5. To stimulate play behaviours, an observer walked 5 metres in front of a camera tripod, displacing birds and creating a space. The birds using the space were then filmed for 5 minutes and the occurrences of frolicking, sparring and food-running were recorded. Undisturbed behaviours, including foraging and locomotion, were determined from video recordings of unenriched areas of the house. Fearfulness of broilers both using enrichments and in unenriched areas was measured using observer avoidance tests in week 5. Walking through and displacing broilers appeared to be a successful method of artificially stimulating sparring and frolicking, with these behaviours observed in 93% of videos, however the presence of enrichments did not have an effect on the level of play recorded ($P > 0.05$). There was also no effect of the presence of enrichments on the activity levels of birds in unenriched areas of the house ($P > 0.05$). Consistent with previous work, levels of

overall activity decreased as broilers aged. In comparison to the control treatment, flight distances in unenriched areas were significantly lower in the perches + dust bath treatment (P = 0.026), and were numerically lower in the perches treatment. This suggests a reduction in fearfulness with increased environmental complexity, and thus possible welfare benefits. We offer support that sparring and frolicking behaviours in chickens may be forms of play, and suggest that further research should investigate whether increasing the level of provision of these enrichments leads to more marked improvements in welfare.

5.1 Introduction

Providing captive animals with environmental enrichment has been shown to improve stereotypical behaviours, reduce fear reactions and increase activity levels (e.g. Beattie et al., 2000; Kells et al., 2001). Increasing the complexity of home environments can also induce “optimism” in animals, which indicates a positive emotional or affective state (Brydges et al., 2011; Douglas et al., 2012). Although broiler chickens are typically raised without environmental enrichment, there is an increasing demand for poultry to be raised to a higher welfare standard. Several studies have demonstrated the positive effects of environmental enrichment on broiler leg health (Bizeray et al., 2002a; Ventura et al., 2010) and activity levels (Kells et al., 2001; Bizeray et al., 2002b). However, very little is known about the influence of these modifications on broiler well-being. One way of investigating the experience of an animal is to measure behaviours associated with positive and negative states, such as fear and play.

Fear is an emotional response to perceived danger and high levels of fear in poultry have been linked to poor performance and a higher risk of injury (Jones, 1996). Chickens also appear to experience a negative emotional state when frightened, and will avoid situations in which they may experience fear (Duncan and Filshie; 1980; Duncan and Petherick, 1991). Provision of enrichments has been shown to reduce fear responses in chickens (Reed et al., 1993) and a reduction in fear could represent an improvement in bird emotional state. Conversely, play has been identified as a positive welfare indicator in animals (Held and Špinka, 2011), and is considered an “opportunistic behaviour” that vanishes from the ethogram when conditions are poor, for example if food becomes less available (Loy, 1970; Fraser and Duncan, 1998; Špinka et al., 2001). Play has been historically defined as any “purposeless motor activity” (Bekoff and Byers, 1981; Bekoff, 1984). More recently, Burghardt (2005) suggested that play behaviour should be spontaneous, apparently self-rewarding, differing from the adult version of the behaviour, repeated in a non-stereotypical way, and occurring in the absence of severe stress. Complex play has been recorded in several avian species, particularly in corvids and parrots (Diamond and Bond, 2003). For domestic fowl, there has been little progress in identifying any play behaviours or investigating their potential use as welfare indicators. However, it has been tentatively suggested by several authors that sparring, frolicking and food-running contain features of play seen in other animals (Kruijt, 1964; Ficken, 1977; Mench, 1988; Duncan, 1998; Cloutier et al., 2004).

As discussed in Chapter 1 (1.6), there are several behaviours exhibited by young broilers that resemble play. Sparring is an immature version of adult fighting that develops in young chicks several weeks before aggressive fighting is seen (Guhl, 1958; Dawson and Siegel, 1967). Although this behaviour was historically recorded as a distinct behaviour from aggression in fowl ethograms (Guhl, 1958; Dawson and Siegel, 1967; Rushen, 1982), it has recently been used as a measure of aggression in juvenile broilers (e.g. Pettit-Riley et al., 2002). Frolicking is an apparently functionless behaviour in young fowl that appears within the first week and is rarely seen after week 10 (Guhl, 1958; Dawson and Siegel, 1967). When frolicking, chicks will perform a spontaneous burst of running, with wing flapping and rapid direction changes (Guhl, 1958 Dawson and Siegel, 1967). An increase in both frolicking and sparring was noted when there was a disturbance, for example a loud noise or turning on the lights (Guhl, 1958; Dawson and Siegel, 1967). Dawson (1962) noted that there was an initial suppression of activity until the perceived danger (loud noise) had passed, and then an abrupt increase in frolicking and sparring. This is consistent with several species that show an increase in play following some environmental disturbance (reviewed in Špinka et al., 2001). Food-running begins to appear during the first week, when a chick picks up rod or “worm” shaped object and runs with it, making loud and repeated peeping noises (Kruijt, 1964). Although food-running may appear to be related to food competition, it occurs even when birds are raised in isolation (Spalding, 1873; Brückner, 1933), before any pursuing response develops (Kruijt, 1964), when birds have ad libitum access to food (Rogers and Astiningsih, 1991; Cloutier et al., 2004), and when birds are given any rod-shaped non-nutritive material, such as pipe cleaners (Rogers and Astiningsih, 1991; Cloutier et al., 2004).

Although the use of perches and straw bales has become common in “higher welfare” housing (e.g. RSPCA, 2017b; M&S, 2015), there are limitations to these current enrichments. For example, the typical single bar perches provided in commercial houses are difficult for broilers to balance on and are infrequently used (LeVan et al., 2000; Norring et al., 2016; Bailie et al., 2018). Straw bales appear to be attractive to broilers, however they mainly function as protected rest areas (Kells et al., 2001; Bergmann et al., 2017; Study 2). Indeed, several materials are more successful than straw at directly stimulating active foraging and dustbathing behaviours in broilers (Shields et al., 2004). Due to the low numbers of enrichments typically provided in commercial housing, it is important that each feature attracts a high level of use and is successful in stimulating an active behaviour. Platform perches, which consist of a large grid that birds can hop onto, appear to be a more attractive to broilers and successful at eliciting perching behaviour in commercial conditions (Bailie et al., 2018). Broilers also show a motivation to perform dustbathing, with a preference for loose friable substrates (Study 1; Chapter 2). Peat moss was particularly successful at eliciting dustbathing in Study 1 (Chapter 2) and attracted use throughout the production cycle. Therefore, peat dust baths and platform perches were considered to be appropriate enrichments that would attract a high level of use and give broilers the opportunity to display a range of highly motivated behaviours. In addition to directly stimulating active behaviours and satisfying natural motivations, it was hypothesised that enrichments would also have a more widespread effect on house activity levels. For example, visual contact with broilers dustbathing may influence birds in unenriched areas (Petherick et al., 1995), and attractive enrichments may encourage locomotion to and from different areas.

Therefore, the main aims of this study were to investigate play behaviours in broiler chickens, and whether sparring, frolicking and food-running would be more prevalent in houses enriched with modifications shown to be attractive in previous trials. As discussed, there is an increase in frolicking and sparring behaviours observed when chickens are disturbed (Guhl, 1958; Dawson and Siegel, 1967). Sparring was also more frequent when birds had access to more space (Hughes and Wood-gush, 1977; Pettit-Riley et al., 2002). These results were supported anecdotally during pilot trials, where it was noticed that when an observer walked through the house, clearing the space behind them of broilers, the birds would run into this space and perform increased frolicking and sparring behaviours. It was therefore hypothesised that an experimenter walking through the birds would stimulate an increase in measurable play behaviours and that this display of positive affective state may be influenced by the presence of environmental enrichment. The behaviour of broilers in unenriched and undisturbed areas was also monitored throughout the production cycle, in order to identify any effect of these preferred enrichments on overall broiler behaviour. The final aim of this experiment was to record the level of use of large dustbathing areas placed along the central line of the house, and determine whether this would be a practical and effective method of creating a dustbathing area in a commercial house.

5.2 Materials and methods

5.2.1 Subjects and housing

A total of 405 000 Ross broiler chickens (Aviagen Ltd) were used in this trial in Northern Ireland, between March and August 2016. The study was conducted over three replicate 6 week production cycles on two commercial farms. Three houses on both farms were used, with all houses matched for structural design and size. Approximately 22 500 birds were placed "as hatched" in each house at the start of each cycle, giving an approximate 50:50 mix of males and females. Chicks of the same strain were placed in all six houses, and the date of chick placement was matched for the three houses on each farm. The houses were standard 19 m x 74 m metal framed sheds, with an average usable floor space of ~1 361 m^2. Stocking densities did not exceed 30 kg/m^2. Their initial bedding material differed, with houses on Farm 1 bedded on straw pellets and houses on Farm 2 bedded on woodshavings at the start of the cycle. Additional woodshavings were distributed across the litter to maintain its condition where necessary on both farms. Natural light was provided through 24 windows with automated shutters along each side of the house. Artificial strip lighting was also provided throughout the cycle, following EU regulations as described in Chapter 2 (2.2.1).

5.2.2 Treatments and experimental design

One house on each farm was allocated to each of three treatments: 1) platform perches (PP), 2) platform perches and dustbathing areas (PP+DB), and 3) control with no enrichment (C). Treatments were allocated to different houses in each of the three replicate production cycles on each farm, such that each treatment was applied to each house over the course of the experiment (Table 12). All enrichments were provided from day 7 of the rearing cycle. The PP treatment contained six ‘platform’ perches, three placed evenly along each long side of the house (Photo 4). These designs have been found to be preferred by broilers in a previous study (Bailie et al., 2018). The platform component of the perches was a plastic grid measuring 2.3 x 0.9 m. Platforms were suspended in a cradle at a height of 20 cm above the litter. The PP+DB treatment contained six platform perches in matching locations to the PP treatment and four dustbathing areas placed along the central line of the house (Photo 4), in order to maximise the number of birds likely to use the dust bath (Chapter 3). The dustbathing areas were contained within steel rectangles measuring 1 x 2.3 m, giving them a total available dustbathing area of 9.2 m^2 per house. The steel rectangle was 7.62 cm high and birds were capable of climbing into the areas within the first week but were not able to perch on the edges. Each dustbathing area was filled with 160 litres of moss-peat (two standard 80 litre bags; Better Growing Ltd, UK), which gave a depth of approximately 5 cm. Dustbathing areas were refilled by researchers twice a week throughout the study. Farmers also examined the dust baths daily and added additional peat once areas of the floor were visible and/or the peat was not considered friable enough for the birds to use.

Table 12. Rotation of treatments presented to broiler chickens over three production cycles, on two farms. Birds were housed with either no enrichment (Control; C), platform perches (PP), or platform perches and dust baths (PP+DB).

	Farm 1			Farm 2		
Cycle	House 1	House 2	House 3	House 1	House 2	House 3
1	C	PP	PP+DB	PP	PP+DB	C
2	PP	PP+DB	C	PP+DB	C	PP
3	PP+DB	C	PP	C	PP	PP+DB

Photo 4. Broiler chickens housed in the platform perches and dust baths treatment (PP+DB). Platform perches (right) were placed along each long side of the house, in matching locations to the perches in the perches only treatment (PP). The dustbathing areas (left) were placed along the central line of the house in the PP+DB treatment.

5.2.3 Data collection

Two researchers visited both farms twice a week during weeks 3, 4 and 5 of each cycle. All filming was performed using Camileo X-Sports cameras, mounted on 1.5 metre high wooden tripods. Filming of general activity and dustbathing areas took place between 09:00 – 13:00 h, filming of play behaviour took place between 13:00 and 15:00 h. All analysis of video footage was performed by the same observer.

5.2.3.1 Play behaviour

On one day per week, play behaviours were recorded in four locations in each house following a walk-through by an observer. Aggressive interactions were also recorded in these observations to monitor the frequency of aggression among broilers following a disturbance, and whether the prevalence of aggressive interactions changed over time. For the purposes of selecting random filming locations, the house was virtually split into 72 sections, using windows and feeder/drinker lines as natural markers, and categorised as either “central” or “edge”. The sections chosen to be filmed each week were randomised using a number table, with the proviso that there were an equal number of edge and central locations. When cameras were positioned they were tilted towards the house floor in between a feeder and drinker line within the chosen section, ensuring a view of at least 2 metres in front of the camera (see Photo 5 for an example camera view). It was impossible to observe an even number of areas around the house that were identically sized, due to the variation in distance between feeder and drinker lines. The largest width between a feeder and drinker was 230 cm in central areas and the smallest was 130 cm in edge areas. However, these areas reflected the open space available in a commercial house and a balanced number of edge and central areas were chosen to account for this variation.

Photo 5. An example camera view after broilers had been displaced by an observer walk-through. The observation area was between the neighbouring feeder and drinker lines, and a distance of 2 m from the camera, which was measured on screen using the distance between three feeder bulbs (2 m).

Once the observer had positioned the camera, they left the house and broilers were allowed to settle for 15 minutes. The observer then re-entered the house and walked directly in front of and away from the camera before turning back and returning to the camera. This displaced the birds and cleared a space a minimum of 5 metres in length in front of the camera. The observer then left the house and each area was filmed for a further 15 minutes before cameras were placed in the next location. The three houses on each farm were filmed at roughly the same time; allowing for walking distance between the houses there was approximately 5 minutes’ difference between the start of filming in each of the three houses. Footage was then analysed using all-occurrence sampling during the 5 minutes after the observer walk-through. The observation area consisted of the space between the feeder/drinker lines and a distance of 2 m from the tripod, which was identified on the screen as the distance between 3 feeder bulbs (Photo 5). With the difference in width between the feeder and drinker lines, depending on the location, this gave an observation area of 2.6 –

4.6 m^2, which was considered during statistical testing. Any occurrences of sparring, frolicking, food-running or aggression were then scored in the five minutes following the birds being disturbed (Table 13). The time after the start of the test was noted for each behaviour and behaviours were grouped by minute (e.g. behaviours performed in minute 1, minute 2) in order to determine whether birds were more likely to perform play behaviours immediately after being disturbed. Data for the four locations were averaged to give one score per house, per week, prior to analysis.

5.2.3.2 Fearfulness

Fear responses were tested in week 5 of each cycle on one farm only (72 broilers in total), by the same observer, using an avoidance distance test based on Graml et al. (2008). In the PP treatment, one bird from four randomly chosen perches and one bird from four random unenriched areas of the house was assessed (a total of eight birds tested). In the PP+DB treatment, one bird from each of four randomly chosen perches, one bird from each of the four dustbathing areas, and one bird from each of four random unenriched areas was assessed (a total of 12 birds). In the C treatment, one bird from each of four randomly chosen areas of the house was assessed (a total of 4 birds). All unenriched areas were balanced for central and edge locations, with random number tables used to choose the locations. When selecting birds for assessment, those on perches or in dust baths had to be more than 20 cm away from the edge of the enrichment, and birds in unenriched areas had to be at least 20 cm away from feeders and drinkers. The avoidance test described by Graml et al. (2008) was validated using laying hens and involves approaching hens from a distance of 1.5 m. The distance between the observer and the bird when they raised their second foot is then measured. For the present trial, there was some concern that a proximity of 1.5 m would be inappropriate for broilers. The birds left within that close a proximity to the observer in a commercial house may have been limited by their leg health and ability to avoid the observer (Vasdal et al., 2018). In pilot trials, it was deemed that a distance of 5 m from a group of broilers could be achieved before there was significant effort from birds in that area to avoid the experimenter. Therefore, during testing the observer approached the chosen location and a bird was randomly selected from a distance of approximately 5 m for assessment, using a numbered Perspex grid and random number tables as in Bailie et al. (2013). The observer slowly approached the chosen bird from a distance of 5 metres, with one hand held in front of the body and the other one loose at the side. At the point when the selected bird withdrew, a line in the litter was made at the toe of the observer's boot, and the approximate distance between the experimenter and where the bird had moved from was recorded in centimetres using a measuring tape. 'Withdrawal' was defined as when the bird lifted its second foot. If the bird failed to withdraw and could be touched an avoidance distance of 0 cm was recorded.

5.2.3.3 General activity

On one day per week, footage of unenriched areas of each treatment was recorded to observe bird behaviour away from enrichments. Two locations away from enrichments, one central and one edge location, were chosen randomly and filmed for half an hour in each house; giving a total of one hour of footage per house. Birds within a 2 m^2 space in the centre of the footage, measured using an overlay on the screen, were included in observations. Scan sampling was used to record bird behaviour within this section. Three scans were performed for each video, at 10 minute intervals following a 5 minute settling period (at 5, 15, and 25 minutes). Broilers were categorised as dustbathing, foraging, sitting inactive, sitting pecking, locomotion (standing or walking), preening, resting or other (Table 13). Each behaviour was expressed as a percentage of the total birds in that scan observation, and scan samples in each location (n total = 377) were averaged for week. Bird density in each scan sample was calculated to account for any variation in results caused by different numbers of birds being counted in each scan sample. For each instantaneous scan, the number of broilers in the observation area was counted and bird density was calculated as the total birds per m^2 and averaged per week.

5.2.3.4 Use of dust baths

On one day per week, two randomly chosen dustbathing areas were filmed for half an hour each, giving a total of one hour of footage per house. Videos were analysed using scan sampling. Six scans were performed per video, every 3 minutes after a 5 minute settling period. The number of birds in the dustbathing area and the number of birds dustbathing was recorded. Dustbathing was defined as birds performing vertical wing shaking or clearly covered in peat and performing side-rubs or prone leg-scratches (van Liere et al., 1991; Table 13). The number of birds dustbathing was then expressed as a percentage of the total number of birds in the dustbathing area.

5.2.4 Statistics

All analyses were performed using IBM SPSS (Version 23).

5.2.4.1 Play behaviour

Overall there were only 9 occurrences of aggression; 4 of these recorded in the C treatment, 3 in the PP+DB treatment and 2 in the PP treatment. No analysis was therefore performed on occurrence of aggressive behaviours. Total play behaviours included occurrences of frolicking, sparring and food-running. There was no significant correlation between observation area size and the total play observed ($r(53) = 0.13$, $P = 0.36$). The residuals for total play behaviours were normally distributed. Cycle did not have a significant effect on total play ($P > 0.05$) and was removed from the model. The main and interaction effects of enrichment and age on the total play behaviours recorded was analysed using general linear mixed models (GLMM) with "treatment" and "week" as fixed factors and "farm" as a random factor. Separate analyses of frolicking and sparring behaviours were also performed using the same model. There were too few incidences of food-running to be included as a separate outcome variable (14 overall; 13 in the PP treatment and 1 in the PP+DB treatment). For the effect of time after the start of the test on total play, the four locations in each house for all treatments were averaged to give the total incidences of play performed per minute. A repeated measures ANOVA with pairwise comparisons was then used to analyse the effect of time on the total play performed after birds were disturbed. This analysis was also performed within-weeks to assess the effect of week on the pattern of play after birds were disturbed. Where a Greenhouse-Geisser correction was applied, adjusted degrees of freedom are reported.

Table 13. Ethogram used to record broiler chicken behaviour (adapted from Guhl, 1958; Kruijt, 1964; Mench, 1988; Girard et al., 2017).

Sparring	A bird simulates fighting behaviour with no obvious aggression or injurious contact. The following behaviours may begin a bout and occur during a bout: jumps with light kicking that make little or no contact with the receiver; stand-offs (threats) in which birds will face up to one another briefly, stepping close to one another and raising their necks to stand practically beak-to-beak (with or without a difference in head height); raising feathers around the neck, usually during a stand-off; stand-off with wing-flapping; stand-off with light pecks at the neck, head or beak of the receiving bird. These differ from aggressive actions in that they are not forceful, prolonged and they do not elicit strong avoidance from the receiver. It would be difficult to estimate a pecking order based on these behaviours. The bird that these behaviours are directed at may or may not respond, in some cases birds attempt a stand-off with a seated bird and are ignored. Birds usually end the short behaviour by sitting down or engaging in another activity.
Food-running	A bird follows and chases (runs at least two paces after another bird to begin the bout) a focal bird that has picked up or obtained a large object that projects from their beak. The focal bird has run from conspecifics but may make rapid and counter-intuitive direction changes towards conspecifics. There are conspicuous peeping noises that typically accompany this behaviour. The bout ends when the bird loses interest and begins another behaviour e.g. sits down or begins feeding.
Frolicking	Spontaneous and rapid running and/or jumping and wing-flapping with no obvious intention, often with rapid direction changes. Running without wing-flapping is not classified as frolicking. A frolicking bout ends when the bird sits down or resumes another activity. Birds displaying frolicking directly leading to sparring are categorised as sparring, to avoid misinterpretation of their movements. Only broilers finishing a frolicking bout within the frame were counted.
Aggression	Aggressive and vigorous pecking and/or kicking where the aggressor makes contact with another bird in a rapid and forceful manner. Aggressive pecking is usually directed at the head of the receiving bird. The receiving bird will take action to immediately avoid the

	aggressor or will respond with aggressive pecking and/or kicking. There is usually a clear 'winner' and 'loser', such that a pecking order could be interpreted. A bout begins when a bird makes forceful contact with another bird, and ends when the bird resumes another activity.
Dustbathing	Broilers were lying and performing head rubbing, vertical wing-shakes, leg scratching, and/or raking the substrate closer to them with their beak. Broilers clearly covered in peat and lying without clearly performing other behaviours were categorised as dustbathing because the end of a dustbathing bout is typically signified by a body-shake which removes excess 'dust'. Broilers preening while covered in peat were classified as dustbathing. Broilers not covered in peat and performing preening without any additional dustbathing behaviours were classified as preening.
Foraging	Scratching and pecking at the ground (from a standing or walking position)
Sitting inactive	Sitting down without performing ground pecking or any other behaviours. The broilers eyes are open and the head is not tucked under a wing.
Sitting pecking	Ground pecking from a seated position
Locomotion	Walking (taking more than one pace in any direction) or standing with no other activity.
Preening	The bird runs their beak through their feathers in a seated or standing position
Resting	The bird sits with its eyes closed, or with its head beneath one wing/ resting on the ground, or the bird lies on one side with or without its eyes closed.

Other	Any other behaviour, including eating and drinking.

5.2.4.2 Fearfulness

Overall flight distance residuals were normally distributed, however equal variance could not be assumed. Due to the small sample sizes, non-parametric tests were used to compare differences between treatments and locations. Kruskal-Wallis tests were applied to test differences between fear responses from broilers in unenriched areas in the three treatments (PP, PP+DB, C; n total = 36) and between the three locations in the PP+DB treatment (floor, dust bath, perch; n total = 36). Comparisons between broiler fear responses on perches in the PP and PP+DB treatments (n total = 24), and between the perches and the floor in the PP treatment (n total = 24) were made using Mann Whitney U tests.

5.2.4.3 General Activity

As the effect that treatment had on each behaviour in unenriched areas of the house was of interest, behaviours were modelled separately, with square root transformations applied where necessary to improve normality. Dustbathing and Other were infrequently recorded during scan sampling and were excluded from analysis. No behaviours (%) were significantly affected by the variables "cycle", "density" and "farm", $P > 0.05$. Analysis of each behaviour was therefore performed using a GLM assessing the main and interaction effects of "treatment" and "age". Where there was a significant effect, Tukey post hoc tests were used to investigate differences.

5.2.4.4 Use of dust baths

In order to investigate whether dust baths continued to attract birds throughout the cycle, the occupancy levels and % of birds dustbathing were analysed by week. Residuals for dust bath occupancy were normally distributed and showed homogeneity of variance, the main effects of week were therefore analysed using a one-way ANOVA with "week" as a treatment factor. The percentage of birds dustbathing showed non-normal distributions and heterogeneity of variance, neither were improved by transformation and a non-parametric Kruskal-Wallis test was applied to analyse the percentage dustbathing by "week".

5.3 Results

5.3.1 Play behaviour

Play was observed in 93% of the videos (n = 217). A total of 2 701 episodes of play were observed across both farms: 1 267 bouts of frolicking, 1 420 birds sparring, and 14 birds engaging in food-running. The highest levels of play behaviour were seen immediately after the broilers had been disturbed by the walk-through ($F_{1.9,102.2}$ = 20.97, $P < 0.001$), with the most play observed in the first minute and then gradually declining (minute 1 = 4.19 ±3.10, minute 2 = 2.96 ±2.25, minute 3 = 2.28 ±2.78, minute 4 = 1.86 ±1.52, minute 5 = 1.29 ±1.26). Significantly more play was performed in minute 1 compared to minutes 3, 4 and 5 ($P < 0.05$), and in minute 2 compared to minutes 4 and 5, and in minute 3 compared to minute 5. There were no significant differences between play performed in minutes 1 and 2, 2 and 3, or 3 and 4 ($P > 0.05$).

There was a significant effect of age on the total play behaviours performed ($F_{2,2}$ = 41.38, P = 0.025), with the lowest average incidence of play behaviour (per 5 minute test period) recorded in week 3 (week 3 = 10.61 ±5.39, week 4 = 13.96 ±7.31, week 5 = 13.15 ±6.91). Age also had an effect on the level of play seen directly after the walk-through (week 3, $F_{4,68}$ = 4.54, P = 0.003; week 4, $F_{2.39,40.68}$ = 16.71, $P < 0.001$; week 5, $F_{1.74,29.60}$ = 36.19, $P < 0.001$), with the pattern of reducing play over time only present from week 4 (Figure 6). In week 3, significantly less play was performed in minute 1 compared to minute 2.

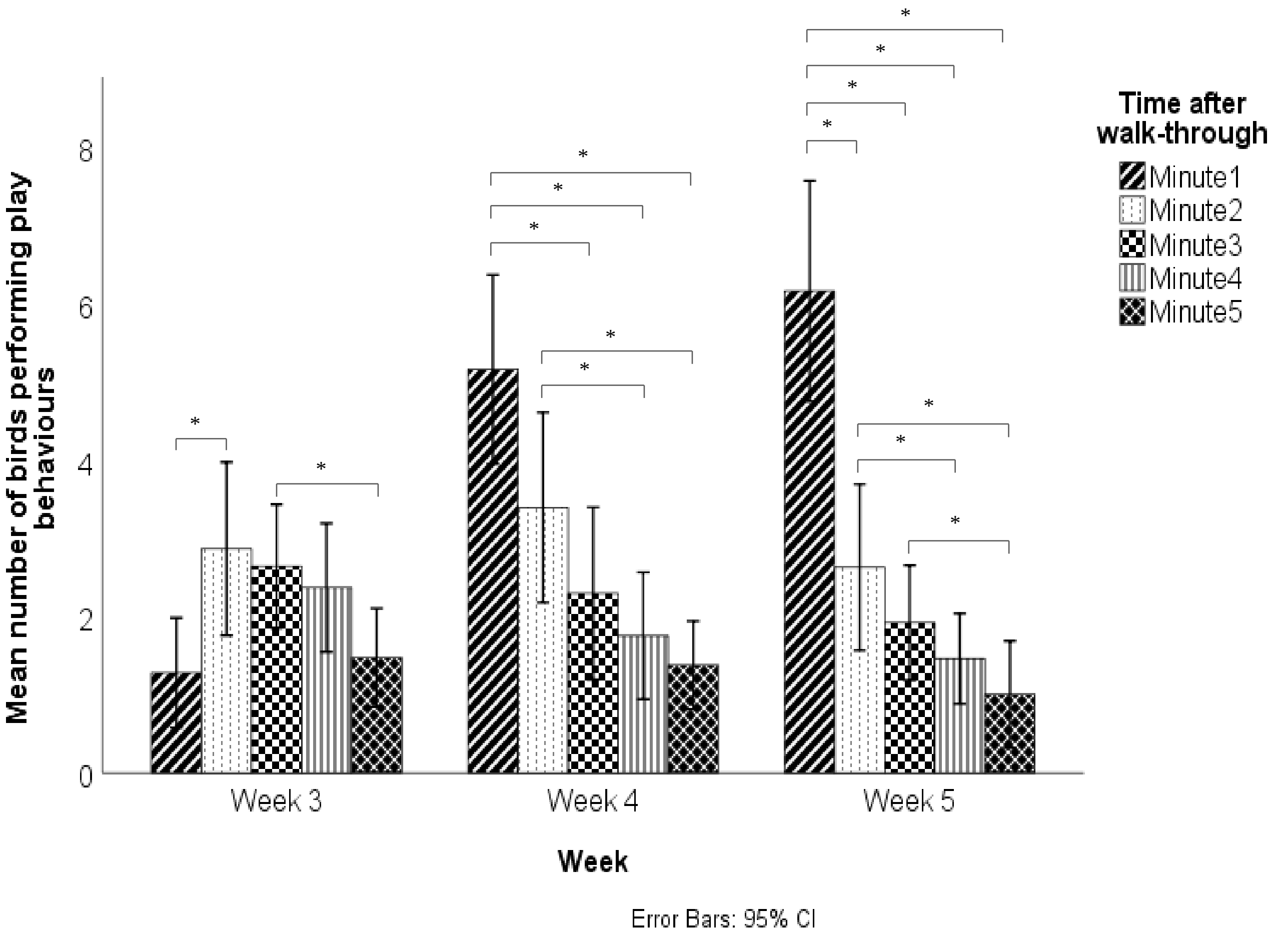

Figure 6. The amount of play recorded in the 5 minutes after broiler chickens were disturbed by a walk-through, in weeks 3,4 and 5 of the production cycle. *denote significance between minutes within weeks.

The presence of enrichments did not significantly affect the average amount of play performed (P > 0.05; PP = 12.97, PP+DB = 13.63, C = 11.13) and no type by week interaction was found (P > 0.05). When analysed separately, levels of frolicking and sparring were not significantly lower in the control treatment compared to either enriched treatments (Figure 7), and there were no significant interactions between treatment and age (P > 0.05). There were also no significant age effects on the average incidence of frolicking per 5 minute test period (week 3 = 5.20 ±5.83, week 4 = 5.96 ±5.43, week 5 = 5.72 ±4.51) or sparring (week 3 = 4.44 ±4.40, week 4 = 6.24 ±6.21, week 5 = 6.50 ±6.05). All play and aggression (sparring, frolicking, food-running and aggression) measured in different treatments are shown in Figure 7.

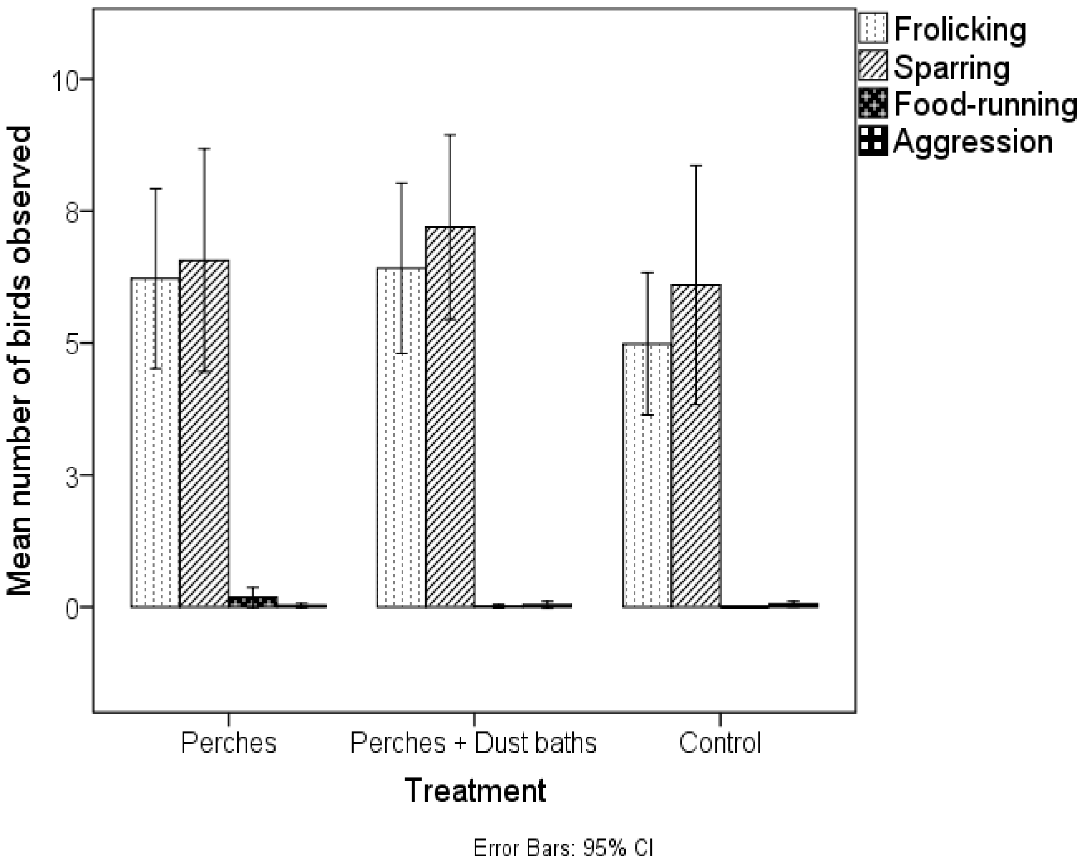

Figure 7. Occurrences of play behaviours and aggressive interactions in broiler chickens recorded in the five minutes after they were disturbed by a walk-through

5.3.2 Fearfulness

Treatment significantly affected the flight distance of birds in unenriched areas of the house (H(2) = 7.27, P = 0.026), with pairwise comparisons showing birds had a shorter flight distance, and could be considered less fearful, in the PP+DB compared to the C treatment (P = 0.033; mean ranks: PP+DB = 14.17, PP = 16.25, C = 25.08; median values presented in Table 14). However, there were no significant effects of location, i.e. whether birds were on the floor or on a perch/in a dust bath, on flight distance in either the PP or PP+DB treatment (P > 0.05; Table 14). There was also no effect of treatment on flight distance of birds on perches in the PP compared to the PP+DB treatment ($P > 0.05$; Table 14).

Table 14. Median withdrawal distance (cm) of broiler chickens from an approaching observer, in houses containing either no enrichment (control; C), perches (P) or perches and dust baths (P+DB). Withdrawal distances were measured in birds in unenriched areas of all treatments, on perches in the P and P+DB treatments, and additionally in dust baths in the P+DB treatment.

	Treatment		
Location	Control (95 % CI)	Perches (95 % CI)	Perches + dust baths (95 % CI)
In unenriched areas	365 (310, 410)	260 (195, 433)	228 (145, 340)
On perches		285 (196, 390)	215 (165, 385)
In dust baths			108 (89, 120)

95% confidence intervals (95% CI)

5.3.3 General Activity

A total of 9679 broilers were observed in unenriched areas and categorised according to Table 13. There were no effects of treatment on any behaviours, however age had a significant effect on the percentage of birds foraging, in locomotion and sitting inactive (Table 15). No incidences of play were recorded during the scan samples. Post hoc tests revealed significantly more birds were foraging and in locomotion (standing or walking) in week 3 compared to week 4 and 5 ($P < 0.05$). Conversely, significantly fewer birds were sitting inactive in week 3 compared to weeks 4 and 5 ($P < 0.05$).

5.3.4 Use of dust baths

Overall, a total of 16 624 broilers were observed in the dust bath, with an average of 58 (±17) birds using each dust bath and 73% (±26%) of them dustbathing. Week had no significant effect on dust bath occupancy ($F_{6,18} = 0.87$, $P = 0.44$). The mean number of birds counted in the dust bath during the cycle was as follows: week 3 = 50.63 ±15.36, week 4 = 63.65 ±19.14, week 5 = 58.90 ±17.21. There was a significant effect of week on the percentage of birds dustbathing (H(2) = 7.45, P = 0.024); ranked means, week 3 = 4.67, week 4 = 12.33, week 5 = 11.50. Pairwise comparisons showed an increase in dustbathing between weeks 3 and 4 (P = 0.039), but no difference in % dustbathing between weeks 3 and 5 or 4 and 5.

Table 15. The effects of enrichment treatment and age on the percentage of broiler chickens performing different behaviours in unenriched areas of the house. Post hoc tests were performed where age effects were significant and are outlined in the results section.

		Perches (CI)	Perches + Dust baths (CI)	Control (CI)	Treatment		Age	
Mean birds (%)	N				F	*P*-value	F	*P*-value
Foraging[1]	18	0.89 (0.37, 1.63)	1.50 (0.78, 2.42)	00.66 (0.23, 1.32)	1.155	0.223	14.34	<0.001***
Locomotion[1]	18	7.63 (5.70, 9.85)	7.66 (5.72, 9.88)	10.31 (8.04, 12.87)	1.910	0.160	11.44	<0.001***
Sit Pecking	18	9.03 (7.08, 10.99)	7.65 (5.70, 9.61)	9.12 (7.16, 11.07)	0.717	0.494	1.643	0.205
Sitting Inactive	18	58.70 (54.71, 62.71)	60.73 (56.73, 64.72)	56.82 (52.82, 60.81)	0.970	0.387	10.62	<0.001***
Preening	18	6.65 (4.99, 8.31)	8.43 (6.77, 10.09)	8.12 (6.46, 9.78)	1.332	0.274	0.521	0.721
Resting	18	14.94 (12.09, 17.79)	11.06 (8.21, 13.90)	11.67 (8.83, 14.52)	2.180	0.125	0.003	0.997

[1]Data were transformed prior to analysis, means and confidence intervals (CI) have been backtransformed to their original scale
*** $P < 0.001$

5.4 Discussion

The main aims of this paper were to explore the effect of increasing environmental complexity on broiler emotional state, measured through levels of play and avoidance behaviours, and whether these enrichments would additionally have an impact on activity levels away from enrichments. Our results suggest that disturbing and displacing the broilers was effective in stimulating certain play behaviours, however the presence of environmental enrichments did not influence the level of play observed. Levels of sitting inactive in unenriched areas of the house were also not affected by the presence of platform perches and dust baths, however birds showed reduced avoidance behaviour when housed with both types of enrichment compared to the barren control. Active behaviours decreased with age in this trial, which is consistent with previous reports of broiler behaviour.

The novel method of disturbing broilers described in this trial appeared to be successful in stimulating sparring and frolicking, with play being performed in 93% of the videos (n = 217). This is consistent with previous studies that report an increase in play following some disturbance to the animals environment (reviewed in Špinka et al. 2001). Specifically for poultry, birds also appear to need a large amount of space to perform sparring behaviours (Hughes and Wood-Gush, 1977; Pettit-Riley et al., 2002). Higher levels of frolicking and sparring were observed immediately after the observer walk-through in the present study, with frequency of these behaviours gradually reducing over time. No play behaviours were observed at all during scan samples of unenriched and undisturbed areas of the house. Although frolicking and sparring may be infrequent in undisturbed areas, it is also likely that scan sampling is an inadequate method of observing these short behaviours. Food-running was only observed on 5 occasions throughout this study, involving 14 birds in total. No specific artificial stimulus was offered in this study to elicit food-running, which has been easily stimulated by previous authors using mealworms and pipe cleaners (Rogers and Astiningsih, 1991; Cloutier et al., 2004). The observer walk-through therefore appears to be a useful method of observing frolicking and sparring only, with additional stimulus needed to provoke food-running.

There was a slightly different effect of age on frolicking and sparring behaviours observed in this study than previously reported. Dawson and Siegel (1967) found that laying hens develop frolicking in week 1 and show an increase in the behaviour until about 4 weeks of age when it declines and is surpassed by sparring behaviours, which peak at around 5 weeks of age and then decline (Guhl, 1958; Dawson and Siegel, 1967). The least of both sparring and frolicking was observed in week 3 in this study, with similar levels of both behaviours in week 4 and 5. It is possible that a different level of sparring and frolicking is seen when birds are given an artificial opportunity to display these behaviours, rather than the normal level of these behaviours in unstimulated areas. However, this finding may reflect the reduced effectiveness of the walk-through method when available space in the house is greater, rather than describing the overall effect of age on play behaviour. In week 3, birds did not immediately use the space created after the walk-through for play behaviours. This may be because broilers had more space overall in the house. As birds grew and space became more restricted, the effect of the walk-through became more pronounced and by week 5, there was an immediate increase in play behaviours in the space created which then declined as broilers settled. It is also possible that young broilers were more fearful, which led to a longer initial period of behavioural suppression before frolicking and sparring occurred. Fearfulness was not measured throughout the cycle in this study, however previous similar research has found that birds were less fearful as they aged (Bailie and O'Connell, 2015).

There was no statistically significant effect of providing enrichments on the total amount of play being performed, or on the level of each individual type of play, although more play was observed in the enriched treatments compared to the control. There was also no effect of treatment on broiler activity levels in unenriched areas. Measures of leg health were also taken during this study and have been published elsewhere (Bailie et al., 2018); these measures were similarly unaffected by treatment. This indicates that any differences in play behaviour were unlikely to be related to physical ability in this study. There has been very little research conducted on the frequency of play behaviours in chickens in different conditions, however these results contradict a previous finding reported by Keeling and Zimmerman (2009). In their trial, small groups of broilers (8 per pen) were housed in either enriched pens (woodshavings bedding, perches and scattered whole-wheat), normal pens (woodshavings bedding only) or barren control pens (no woodshavings or enrichment). Birds were then given “toys” (plastic toothpicks, a ball, a cardboard box) to try to stimulate play. Contrary to their expectations, they found that birds spent less time playing in enriched conditions compared to the normal and barren treatments. This may be because play is an inaccurate measure of positive emotions, however the toys offered may also have had little biological relevance and therefore were not suitable for stimulating sparring and frolicking. It may also be that perch provision had reduced the space available for birds to perform play. Hughes and Wood-Gush (1977) found that laying hens need a considerable amount of space to display sparring behaviours, and several recent studies have found a reduction in sparring when broilers were housed with perches (Pettit-riley et al., 2002; Ventura et al., 2012). When comparing various perch types, Ventura et al. (2012) found the most sparring was performed when broilers were housed with either no perches or perches that took up the least floor space. Our observations of sparring and frolicking are supportive of previous authors that suggest these behaviours resemble play. Frolicking appeared to be spontaneous and purposeless, and there was usually a clear distinction between sparring and aggressive interactions. Recent studies have hypothesised that a reduction in sparring in juvenile broilers leads to an improvement in welfare (Pettit-riley et al., 2002; Ventura et al., 2012). Further research investigating the motivation and frequency of these behaviours will be essential in determining how they may be employed as indicators of animal welfare.

Fear is an adaptive response, however it is associated with negative welfare and can cause poor performance, injury and death in commercial conditions (Duncan and Filshie, 1980; Jones, 1996). In the present trial, birds housed in the barren control treatment had significantly longer flight distances compared to those housed with perches and dust baths, and numerically longer flight distances to those housed with perches only, which suggested birds in the most complex environment were less fearful. This is consistent with previous studies that have reported reduced fear levels in enriched environments, probably as a result of young birds being exposed to varied and novel stimuli that do not all require a fear response (Jones and Waddington, 1992). There is also some evidence that dustbathing behaviour is linked to fearfulness (Gerken et al., 1988; Vestergaard et al., 1993). However, no difference in fear response was found when birds were using an enrichment (in the dust bath or on a perch) compared to those on the floor. The anti-predator hypothesis suggests that birds on elevated perches are more protected from ground predators and will be less vigilant (Newberry et al., 2001), which implies that birds on perches would be slower to show a fear response than those on the ground. It may be that the perches were too low to the ground to make a difference to behavioural responses, however birds do not appear show a difference in vigilance behaviour depending on the height of a perch (Brendler et al., 2014). Broilers using the dust baths were at floor level and so a difference in fear levels as a function of vigilance was less expected.

Consistent with previous studies, a high number of broilers were attracted to the peat dust baths and a high percentage of them were using the peat for dustbathing (Petherick and Duncan, 1989; de Jong et al., 2005; Study 1). As expected, the amount of foraging and locomotion decreased as birds aged in unenriched areas of the house (Weeks et al., 2000; Bessei, 2006). However, contrary to our prediction there was no effect of treatment on these behaviours, suggesting that although enrichments were attractive, they did not influence overall activity levels. Kells et al (2001) found that a high provision of straw bales increased activity in unenriched areas of the house. More recent research that used a density of bales that more closely resembled commercial practices did not find a similar increase in active behaviour (Bailie et al., 2013). It may be that enrichment density had a similar impact on this trial, and that a higher number of dust baths and perches would result in a more widespread effect on house behaviour. There is generally a limit to the number of enrichments that can practically be provided on commercial farms, however more information on the optimal level of enrichments would be valuable. Peat was used in this trial due to its attractiveness as a dustbathing substrate (Petherick and Duncan, 1989; de Jong et al., 2005; Study 1), however it is expensive and not an environmentally sustainable option for a commercial enrichment. In this thesis, ground oat hulls have been suggested as an alternative dustbathing substrate for commercial housing and future work on the optimal level of enrichments should include substrates compatible with intensive systems.

One aim of this study was to consider whether providing large rectangular, steel dustbathing areas along the central line of the house would be an appropriate method of introducing a dustbathing enrichment to commercial housing. There was an average of 58 birds in each peat dust bath, with approximately 73% of those birds dustbathing. This is a significantly larger number than those present and dustbathing in the rings of oat hulls described in Chapter 3. However, the total dustbathing area available for broilers was similar; 8.6 m^2 available with oat hull rings and 9.2 m^2 available with peat baths. With four large dustbathing rectangles of peat, there would be an average of 232 birds using the dustbathing areas, and 169 dustbathing (based on 73%). In Chapter 3, there was an average of 11 birds using each oat hulls dustbathing ring, and 24% of those dustbathing. With 9 rings placed around the house, this equates to around 99 birds in total at any one time in the rings, and 24 birds dustbathing. While the study in Chapter 2 has shown that peat is a more attractive dustbathing substrate than oat hulls, only 28% of broilers in peat rings were observed dustbathing at any one time. It is possible that the dustbathing area design may also have influenced the prevalence of dustbathing. It has been suggested that dustbathing is socially facilitated (Vestergaard et al., 1990; Duncan et al., 1998), and that the sight of a dustbathing bird stimulates dustbathing in other birds (Duncan et al., 1998). Chickens will synchronise their dustbathing to include the entire group, which may reduce the risk of individual predation during performance of this vulnerable behaviour (Wood-Gush, 1989, in Olsson et al., 2002; Lundberg and Keeling, 2003). Placing the dustbathing material in a larger area may have facilitated group dustbathing, with more broilers being stimulated to dustbathe by the sight of dustbathing conspecifics. However, several recent studies have failed to show that chickens will increase their dustbathing when presented with a dustbathing conspecific (Olsson et al., 2002), or that the social facilitation may be connected to rank (Lundberg and Keeling, 2003). It is also likely that the peat was a more successful dustbathing material and this result may not be repeated with oat hulls. Further research comparing large and small dustbathing areas would be needed in order to confirm any effect of social facilitation. In terms of practicality, these larger areas were less time-consuming to maintain and clean between cycles, and are likely to be a more practical design for commercial housing.

5.5 Conclusions

Disturbing the broilers and creating space appeared to be an effective method of stimulating frolicking and sparring, and may be a suitable method for investigating these behaviours further. Additional research into the normal levels of these behaviours in commercial broiler housing would be valuable. The provision of dust baths and platform perches at the level of provision in this study did not significantly affect the amount of play performed, or the activity levels in unenriched areas of the house. However, there was a reduction in apparent fearfulness observed when birds were provided with both types of enrichment, compared to the barren control, which suggests the enrichments may have had a positive effect on bird welfare. Providing broilers with large steel bordered dustbathing areas was more successful in eliciting dustbathing and a more practical method than distributing smaller rings, and may be a suitable method for creating dustbathing areas in intensive housing. It is suggested that the motivation for sparring should be carefully considered before classifying the behaviour as aggression, and that more research is needed to determine whether play behaviour would be a suitable measure of positive emotion in poultry.

Chapter Six

The role of environmental enrichment in improving broiler chicken welfare

In general, changes to animal welfare standards in the UK reflect societal concerns (Caporale et al., 2005; Vanhonacker and Verbeke, 2014), and the general public have shown a distaste for barren intensive housing (Vanhonacker et al., 2008; Verbeke, 2009). Retailers and broiler producers have responded to these concerns by developing alternative rearing systems. These systems contain environmental enrichments that aim to reduce the prevalence of painful conditions and provide broilers with an opportunity to express natural behaviours. There is currently a lack of research capable of providing evidence-based recommendations to producers and policy makers about optimal enrichments. New and innovative housing designs for laying hens are currently being developed in the EU. For example, government funded Dutch scientists proposed a new form of indoor laying hen housing that include transparent side areas for dustbathing, allowing hens to dustbathe and sunbathe in natural light on a substrate that is constantly refreshed by a conveyor belt (van Weeghel et al., 2016). Rondeel laying hen housing systems have now been in use in the Netherlands for 7 years (van Niekerk and Reuvekamp, 2011; Waninge, 2016). These adapted barn systems have day and night areas, access to natural light, dust baths of peat, a woodchip floor and a visitor tunnel for consumers to view the birds (van Niekerk and Reuvekamp, 2011). These systems are expensive to create and are dependent on a positive consumer response. Progress in broiler chicken “higher welfare” housing has been limited by a lower understanding of broiler welfare problems among the general public (EU Commission, 2000) and by broilers showing a lack of interest in enrichments currently provided (Rodriguez-Aurrekoetxea et al., 2015; Arnould et al., 2004). However there remains a demand in the UK for high welfare meat, and producers show an interest in further developing a competitive product for this market. In addition, broilers show a motivation to perform natural behaviours that are not accommodated for in barren housing, and more complex environments may improve issues such as low activity levels and poor leg health (Kells et al., 2001; Bizeray et al., 2002a; Ventura et al., 2010). This thesis describes four studies performed in commercial broiler housing on the effectiveness of various enrichments and enrichment combinations on broiler behaviour, leg health, and affective state.

Dustbathing is an adaptive behaviour in red junglefowl that has persisted in a similar pattern in their domestic fowl descendants (Vestergaard et al., 1990; Schütz and Jensen, 2001). A high motivation to dustbathe (de Jong et al., 2007; McGrath et al., 2016), signs of frustration when thwarted (Vestergaard et al., 1997; Zimmerman et al., 2000), and a rebound effect of substrate deprivation (Hughes and Duncan, 1988; Vestergaard, 1982; Vestergaard et al., 1999) have been reported in laying hens. There is a lack of similar research performed using broilers chickens, which have diverged from laying hens in their genetics, behaviour and housing (Wise, 1970; Bessei, 2006; Weeks et al., 2000; Lay et al., 2011). However, some authors have argued that broilers show similar patterns of dustbathing and are likely to be similarly motivated (Vestergaard and Sanotra, 1999). When housed in cages and given temporary access to a dustbathing substrate, many broilers will perform dustbathing every day (Stub and Vestergaard, 2001) or every other day (Vestergaard and Sanotra, 1999). Small scale studies largely looking at substrate preferences have shown that broilers will use a dustbathing material if offered, and identify sand as an attractive substrate (Vestergaard and Sanotra, 1999; Shields et al., 2004; Toghyani et al., 2010; Villagrá et al., 2014). However, dustbathing is often considered an infrequent and unimportant behaviour in modern broilers (Murphy and Preston, 1988). Several laboratory studies have found that the percentage of broilers observed dustbathing at any one time was extremely low, with an average of 0.2 – 1% (Weeks et al., 2000; Kristensen et al., 2007; Alvino et al., 2009; Schwean-Lardner et al., 2012a). A similar low prevalence has been reported at a commercial level in broilers housed on woodshavings (0.18%; Bailie et al., 2013) and straw pellets (0.3-0.46%; Bergmann et al., 2017). Therefore, a priority of Study 1 (Chapter 2) was to determine whether broilers would use a dustbathing substrate if it was offered, and whether they displayed any substrate preferences at a commercial level. While a preference for peat as a dustbathing substrate has been demonstrated in laying hens (Petherick and Duncan, 1989; de Jong et al., 2007), Study 1 appears to be the first experiment to show that broiler chickens find peat similarly attractive compared to other bedding materials. This provided justification for its inclusion in Study 4 (Chapter 5), which sought to provide optimal enrichments for broilers. During Study 1, a similarly low prevalence of dustbathing was found in broilers using areas with litter (0.72%), woodshavings (0.49%) and straw pellets (1.79%). However, those using alternative substrates showed substantially higher levels of dustbathing, with 28% in peat performing dustbathing, and 19% in oat hulls. This high level of dustbathing was also seen in larger areas of peat in Study 4 (Chapter 5), with more birds observed throughout the study and an average of 73% of them dustbathing at any one time. These results suggest that low levels of dustbathing previously reported may have been confounded by a lack of appropriate substrate. Broilers readily used peat and oat hulls, and their levels of dustbathing increased as they aged in these substrates but not in woodshavings, litter, or straw pellets (Study 1, Chapter 2).

Demonstrating that commercially housed broilers will make use of a dustbathing substrate when offered, and show substantially higher levels of dustbathing in substrates that they are not typically bedded with, identifies a possible way in which their welfare could be improved. Preventing frustration, which is considered to be a form of suffering, associated with dustbathing deprivation would reduce an aspect of negative welfare (Duncan, 2005). Widowski and Duncan (2000) argue that rather than preventing suffering, dustbathing may be a self-rewarding opportunistic behaviour that increases pleasure. Even if this is the case, providing a dustbathing substrate to broilers would improve their quality of life and may act as an indicator for positive welfare. While peat and sand appear to possess qualities that make them attractive dustbathing substrates (Study 1; Shields et al., 2004), their use in commercial housing is unlikely. Sand could not be included in Study 1 because it would have interfered with the litter disposal process, and although peat is a common bedding in other European countries (Kaukonen et al., 2017a,b), it is considered environmentally unsustainable in the UK (Defra, 2010; The Guardian, 2012). In Study 1 (Chapter 2), oat hulls were also tested as a potential dustbathing substrate, and appear to be successful in promoting dustbathing and foraging in broilers. This material would be safe for broilers (Hetland and Svihus, 2001) and would increase the value of a farming by-product. Oat hulls may offer additional benefits to producers by stimulating exercise in broilers, which may improve leg health (Reiter and Bessei, 1995). No change in final body weight was noted and there was no increase in general activity levels observed in Study 3 (Chapter 4) when broilers were provided with oat hulls or a combination of oat hulls and straw bales. However, in both cases, provision of this dustbathing substrate led to an improvement in gait score compared to an unenriched house. This suggests inclusion of a suitable dustbathing area may improve leg health, which would improve broiler welfare by reducing the pain and risk of death associated with leg disorders. Furthermore, a reduction in leg culls would have financial implications for commercial producers, and provision of a dustbathing substrate may be a competitive method of discriminating their product as high welfare.

Despite straw bales being an almost ubiquitous enrichment in higher welfare broiler systems, there appears to be very little research exploring their use. Several studies have found an inconsistent effect of straw bales on overall activity levels, due, in part, to their substantial differences in methodology (Kells et al., 2001; Bailie et al., 2013; Bailie and O'Connell, 2014; Bergmann et al., 2017). In particular, it is worth noting the variety of enrichment bales that are currently supplied to higher welfare housing (Photo 6). Kells et al. (2001) and Bergmann et al. (2017) observed farms supplied with long-cut traditional straw bales (Photo 6), which are demolished slowly by broilers or not at all. This allows high numbers of bales to be easily maintained across the production cycle and gives broilers a stable area to perch on. Only one study has looked exclusively at the effect of long-cut straw bales on broiler behaviour (Kells et al., 2001). In their experiment, there was a significant reduction in the amount of time broilers spent sitting and resting when housed with a high density of these bales, compared to barren housing. Bergmann et al. (2017) also found less lying behaviour in broilers reared with straw bales, however these birds were also reared with a lower stocking density, access to an outdoor run, and additional perches and pecking objects. Both authors found that broilers would primarily use the bales as resting areas to cluster around (Kells et al., 2001; Bergmann et al., 2017), with 51% of broilers observed resting around straw bales at the beginning of the cycle (Bergmann et al., 2017). Recently, the use of plastic wrapped short-cut straw bales in place of traditional long-cut bales has become common (Photo 6), especially in Northern Ireland. These bales are considered by some to be more biosecure and practical for commercial farms (personal communication). The plastic on the bales is cut open, and broilers peck and scratch at the straw until the bale collapses (Photo 6). The loose, dry chopped straw improves litter condition in the immediate vicinity and farmers can leave the bales in wet areas for the broilers to "self-bed" (personal communication). Broilers readily dismantle these bales, especially in later weeks, suggesting they are an attractive and interactive enrichment. However, this rapid degradation results in an unstable platform for perching and means that there are few enrichment bales left by the end of the cycle. With a provision of 2 bales per 1 000 birds, which is higher than the RSPCA requirements (RSPCA, 2017a), farmers tend to place all bales in the house and cut them open in a staggered manner, or introduce a smaller number that are then replaced throughout the cycle (as described in Study 2, Chapter 3). No effect of these bales on activity levels has been found (Bailie et al., 2013), even when the level of provision was increased (Bailie and O'Connell, 2014). However, broilers latency to lie was longer when they were housed with bales, which could indicate an improvement in leg health (Bailie et al., 2013). There was a similar failure for plastic wrapped straw bales to influence activity levels in Study 2 (Chapter 3), and no effect of bale provision on gait score. This appears to be the first study to provide information on the behaviour of broilers directly around plastic-wrapped bales. Of the broilers observed within a 0.4 m area around the bale, 50% were sitting down or resting (Study 2, Chapter 3). This is consistent with levels of resting seen around traditional straw bales (Bergmann et al., 2017), and suggests they are equally suitable in this regard. Only 5% of broilers were observed pecking at and foraging around plastic-wrapped straw bales during Study 2, which is comparable with the pecking directed at long-cut straw bales (6%; Bergmann et al., 2017). However, in Study 3 (Chapter 4), there was an average of 310 pecks directed at two straw bales over a 10 minute focal period. All bales were also dismantled by the end of the study, suggesting their use as a pecking and scratching enrichment should not be overlooked. Overall, it appears that both long-cut straw bales and plastic wrapped straw bales offer attractive areas of protective cover that provide broilers with an area to rest. The levels of foraging and pecking around each bale type may be similar; however, it is difficult to draw direct comparisons between such varying studies. Natural light has been found to have a large influence over activity levels (Bailie et al., 2013), and was supplied to all broilers in Study 2 and 3, which may have reduced the impact of straw bales reported previously in non-windowed housing (Kells et al., 2001; Bergmann et al., 2017). However, the increase in activity levels seen with long-cut straw bale provision has yet to be repeated with plastic-wrapped bales, and a direct comparison of these enrichment types will be needed in order to make appropriate recommendations for bale enrichments. There also appear to have been no studies performed on the use of Miscanthus bales, which are similarly permitted under the RSPCA Assured standards (CIWF, 2013).

Photo 6. Examples of the types of enrichment bales used in higher welfare broiler housing. The RSPCA require 1.5 straw (top) or Miscanthus (centre) bales per 1 000 chickens to be maintained at all times (photos reproduced with permission from Compassion in World Farming; CIWF, 2013). Short-cut plastic wrapped straw bales (bottom) are commonly used in enriched housing in Northern Ireland and were used in Study 2 and 3 (Chapter 3 and 4)

Environmental enrichments are usually designed to satisfy particular motivations or allow expression of a natural behaviour, for example perches are included solely to encourage perching behaviour. However, a broader aim of enrichment is to improve the complexity of the environment. This is often achieved by including a variety of features to encourage exploration and activity (Newberry, 1999). As discussed, when both straw bales and oat hulls were provided during Study 2 (Chapter 3), there was an improvement in broiler walking ability and no negative effect on final body weights. There was also no difference in the way the two enrichments were used when provided together rather than individually (Study 2), but a large difference in the behaviours performed with each enrichment. More foraging and dustbathing was seen in oat hulls, while straw bales were primarily used for rest. This suggests addition of both enrichments would satisfy separate motivations and allow for a range of behavioural expression, without compromising production levels. If a dustbathing material is to be introduced as a supplementary enrichment, then identifying the most attractive way of presenting multiple enrichments will help maintain a high level of use. This was explored in Study 3, in which straw bales, oat hulls and a pecking chain enrichment were arranged in different combinations around a commercial house (Chapter 4). It was predicted that creating complex areas with multiple enrichments would attract more broilers and increase the overall use of each enrichment. However, there was no difference in enrichment use when each feature was presented singly compared to in various combinations with other enrichments. The amount of sitting pecking was the only behaviour to be affected by enrichment combination, with more seated pecking performed in oat hulls when combined with pecking chain. It is difficult to clearly identify the cause for this, visual contact with broilers pecking at the chains may have stimulated more pecking in the oat hulls or encouraged more pre-dustbathing behaviour (Guy and Wright, 2003). There was a higher level of interest in a pecking enrichment than previously reported (Arnould et al., 2004), and more research would be needed to clarify any potential benefits for commercial housing. Practically, there were issues with confining short-cut straw bales to a particular house section. There was a build-up of dry straw in specific areas as bales were dismantled, and farmers were unable to use bales as a way for broilers to "self-bed" wet patches of litter. Study 3 was a short trial, and a starting point for future research, however there appeared to be no obvious benefits to grouping enrichments together.

The role that perches have in improving broiler housing is unclear. While poultry show a strong motivation to perch and experience frustration when thwarted (Olsson and Keeling, 2000; Olsson and Keeling, 2002b), broiler chickens are unable to make use of normal perching opportunities. A high body weight and peripheral centre of gravity has limited their ability to jump up and balance on single bars, which has led to a low level of use of these perches (LeVan et al., 2000; Rodriguez-Aurrekoetxea et al., 2015; Norring et al., 2016; Bergmann et al., 2017). Single bar perches are often totally unoccupied in commercial housing and farmers tend to be dismissive of their inclusion (personal communication). Recent studies have shown that broilers will make use of elevated platforms, which provide them with a raised flat area that requires little balancing (Norring et al., 2016; Bailie et al., 2018). These platforms are more attractive to broilers and may be a more appropriate method of enabling their natural perching behaviour (Bailie et al., 2018). Satisfying a highly motivated behaviour is likely to improve broiler mental well-being (Duncan, 1998), and the inclusion of a feature that promotes natural behaviour will be appealing to consumers (Verbeke, 2009). In addition, it has been suggested that introducing perches may provide broilers with an opportunity to exercise, which could improve leg health, walking ability and bone quality (Reiter and Bessei, 1995; Bizeray et al., 2002a; Ventura et al., 2010). No support for this theory was found during Study 4 (Chapter 5), in which broilers were housed with either platform perches or platform perches and dust baths. Broilers showed no increase in activity levels in these enriched conditions compared to a barren control, which is consistent with previous work showing no improvements in walking ability when commercially housed broilers were provided with bar perches (Bailie and O’Connell, 2015). Measures of gait score, severity of leg deformities, footpad dermatitis and production parameters were also taken during Study 4 and have been reported elsewhere (Bailie et al., 2018). There appeared to be no beneficial effect of providing platform perches on any of these additional leg health measures (Bailie et al., 2018), which offers conflicting results to a recent Finnish study. In this study (Kaukonen et al., 2017a), the authors report a significant improvement in gait score and the incidence and severity of tibial dyschondroplasia when broilers were housed with platforms. This inconsistency is likely to be due to the substantial differences between these two studies. The commercial houses used by Kaukonen et al. (2017a) were significantly smaller (floor area of 337 to 797 m^2 compared to 1 361 m^2 in Study 4) and housed fewer birds (5 016 to 13 947 compared to 22 500 in Study 4), but had higher stocking densities (36 to 43 kg/m^2 compared to 30 kg/m^2 in Study 4). Their broilers were also raised entirely on peat rather than woodshavings, as is the norm on Finnish farms (Kaukonen et al., 2017a). Most importantly, their raised platforms covered 10% of the floor area available and birds could use space under the platforms, while in Study 4 the total area of platforms represented less than 1% of the total floor area (2.07 m^2 per platform, with 6 platforms) and broilers were not able to walk underneath them. Any one of, or a combination of, these factors may have led to the improvement in leg health not seen in Study 4 (Chapter 5), and more research looking at different designs and particularly at different levels of perch provision will be needed.

It has been widely accepted that animals are sentient creatures and ‘experience’ their physical state and environment in some way (Duncan, 2006). Although welfare measures are commonly resource-based or dependent on physical health, a separate recognition of an animal’s cognitive needs is common in definitions of welfare. Indeed, Duncan and Petherick (1991) argue that animal welfare should depend solely on the animal’s mental state, because if its psychological needs are met then this will generally cover its physical needs, i.e. if an animal ‘feels’ well then it is likely to be physically well. Studies that draw conclusions about broilers mental experience are almost exclusively related to negative welfare parameters, often measured through inference depending on their physical health and related behaviours. For example, leg disorders are assumed to be painful, and broiler walking ability or the presence of infection can be used to imply level of suffering (Weeks et al., 2000; Bradshaw et al., 2002; Gentle, 2011). Fearfulness and frustration, resulting from exposure to negative stimuli or lack of resources, can also be assessed using behavioural measures (Jones, 1996; Duncan, 1998; Olsson and Keeling, 2000; McGrath et al., 2016). The assumption of these methods is that a lack of response or a reduction in the severity of response will indicate the absence of suffering or an improvement in welfare, respectively. In Study 4 (Chapter 5), a reduction in fear response was observed in broilers housed with dust baths and platform perches, compared to those in the barren control. Environmental enrichment has previously been shown to reduce fearfulness in a range of species, possibly by preventing animals from always associating novelty with danger (Jones, 1996). When laying hens are provided with a more complex environment they show a range of behaviours associated with reduced fear, including attenuated avoidance of an observer (Jones and Waddington, 1992) and reduced reactivity during depopulation (Reed et al., 1993). Although the adapted avoidance tests used in Study 4 were only performed in one week and should be interpreted with care, it is possible that environmental enrichment reduced this measure of suffering. However, the main purpose of Study 4 was to explore whether environmental enrichment would provide broilers with an element of positive welfare, by inducing positive affective states and pleasure. Focusing on providing animals with a good quality of life rather than reducing their level of suffering has gained traction recently (FAWC, 2009; Wathes, 2010), however little progress has been made in poultry and there are no clear indicators of positive welfare in broilers. Play is considered to be a self-rewarding “opportunity behaviour” and a generally reliable indicator of positive welfare in other animals (Burghardt et al., 2005; Špinka et al., 2001). It was argued during Chapter 1 (1.6), that sparring and frolicking behaviours displayed by domestic fowl resemble forms of play, and that these may be useful in further investigating broiler emotional state. Levels of sparring are typically low in broilers (Weeks et al., 2000; Pettit-Riley et al, 2002), probably due to an overall reduced motivation to perform active behaviours. However, a novel method of stimulating frolicking and sparring was developed in Study 4 (Chapter 5). A walk-through by an observer created an open space among broilers and caused a physical (but not severely frightening) disturbance, which stimulated sparring and frolicking behaviours in 93% of tests. This method may prove useful in further investigating the motivation and welfare associations of these behaviours. Although no conclusions can be drawn within this study about the self-rewarding nature of frolicking or the relationship between aggression and sparring, both behaviours appeared to be stimulated by factors that promote play in other species (Špinka et al., 2001). However, levels of frolicking and sparring were not significantly influenced by provision of dust baths of peat and/or platform perches (Study 4; Chapter 5). This was contrary to our prediction that environmental enrichment would increase this measure of positive welfare. It may be concluded that play is a poor indicator of positive welfare, or that the environmental enrichment provided was not sufficient to induce positive welfare. Nevertheless, as broilers were more fearful and tended to perform less play-like behaviours when housed in barren conditions, it is possible that environmental enrichment is capable of influencing broiler mental state independent of their physical abilities. A recent report on broiler welfare found that there were no peer reviewed articles available on the importance of play behaviour and sunbathing in young broilers (Hoeks et al., 2011). The Welfare Quality assessment protocol for broilers include two measures designed to assess broiler mental well-being; avoidance testing, which has been only validated with laying hens, and qualitative behaviour assessment (QBA; Welfare Quality, 2009). It is clear that research into positive welfare is in its infancy for poultry. While broilers will make use of attractive environmental enrichments when offered, future studies are likely to give interesting insight into broiler emotional state and whether these additions will be capable of providing intensively farmed broilers with a “life worth living”.

General Conclusions

Broiler chickens will make use of an attractive dustbathing substrate in commercial housing and continue to dustbathe throughout the production cycle (Study 1 and 2). In agreement with previous research involving laying hens (Petherick and Duncan, 1989), broiler chickens show a preference for moss-peat over other friable materials for foraging and dustbathing (Study 1). Broilers also appeared to identify oat hulls as a dustbathing substrate, with significantly more dustbathing performed in oat hulls compared to standard litter, woodshavings and straw pellets (Study 1). This material is a by-product of oat milling and may be suitable for inclusion in commercial housing. In addition, provision of dust baths of oat hulls, both individually and in combination with straw bales, resulted in an improvement in broiler gait score compared to the barren control (Study 2). This suggests that a dustbathing enrichment may function to encourage exercise and improve leg health, in addition to allowing expression of a natural behaviour. However, no increase in activity levels was seen in unenriched areas of the house when broilers were provided with dust baths (Study 2 and 4) or with platform perches (Study 4), and indeed there was a counterintuitive reduction in activity in some enriched conditions (Study 2). This demonstrates the difficulties involved in increasing overall house activity, and may result from a low density of enrichments or from broilers using unenriched areas primarily for rest.

A direct comparison of oat hulls and plastic-wrapped straw bales found that bales tend to be used as protected areas for rest, while oat hulls attracted relatively high amounts of dustbathing and foraging (Study 2). It is therefore suggested that oat hulls may satisfy a separate motivation to straw bales and could be introduced as a supplementary enrichment. However, there appear to be no benefits to grouping straw bales and oat hulls together into “enrichment areas”, and practical benefits to presenting them separately (Study 3). Similarly, presenting a pecking chain individually compared to in various combinations with oat hulls and straw bales did not influence its level of use, although there was an unexpectedly high overall interest in the pecking enrichment (Study 3). Frolicking and sparring, which are behaviours that resemble play, were successfully stimulated by an observer walk-through that created a physical disturbance and area of open space (Study 4). Contrary to our prediction, broilers housed with dust baths of peat and platform perches did not show significantly more of these play behaviours, although less of both were observed in the barren control housing (Study 4). The enriched housing did however appear to result in less fearfulness, which may indicate an improvement in emotional state.

This research has shown that providing a dustbathing substrate to broiler chickens may have multiple benefits, including improving leg health, reducing fearfulness and allowing the expression of a natural behaviour. Additional commercial scale research with varying levels of enrichment provision will be needed to further explore these findings. In addition, the existence of possible play behaviours in poultry has been discussed, and a novel way of stimulating frolicking and sparring has been outlined. Research into positive welfare indicators in poultry is sparse, and these ideas may be useful in future investigations of broiler mental state.

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